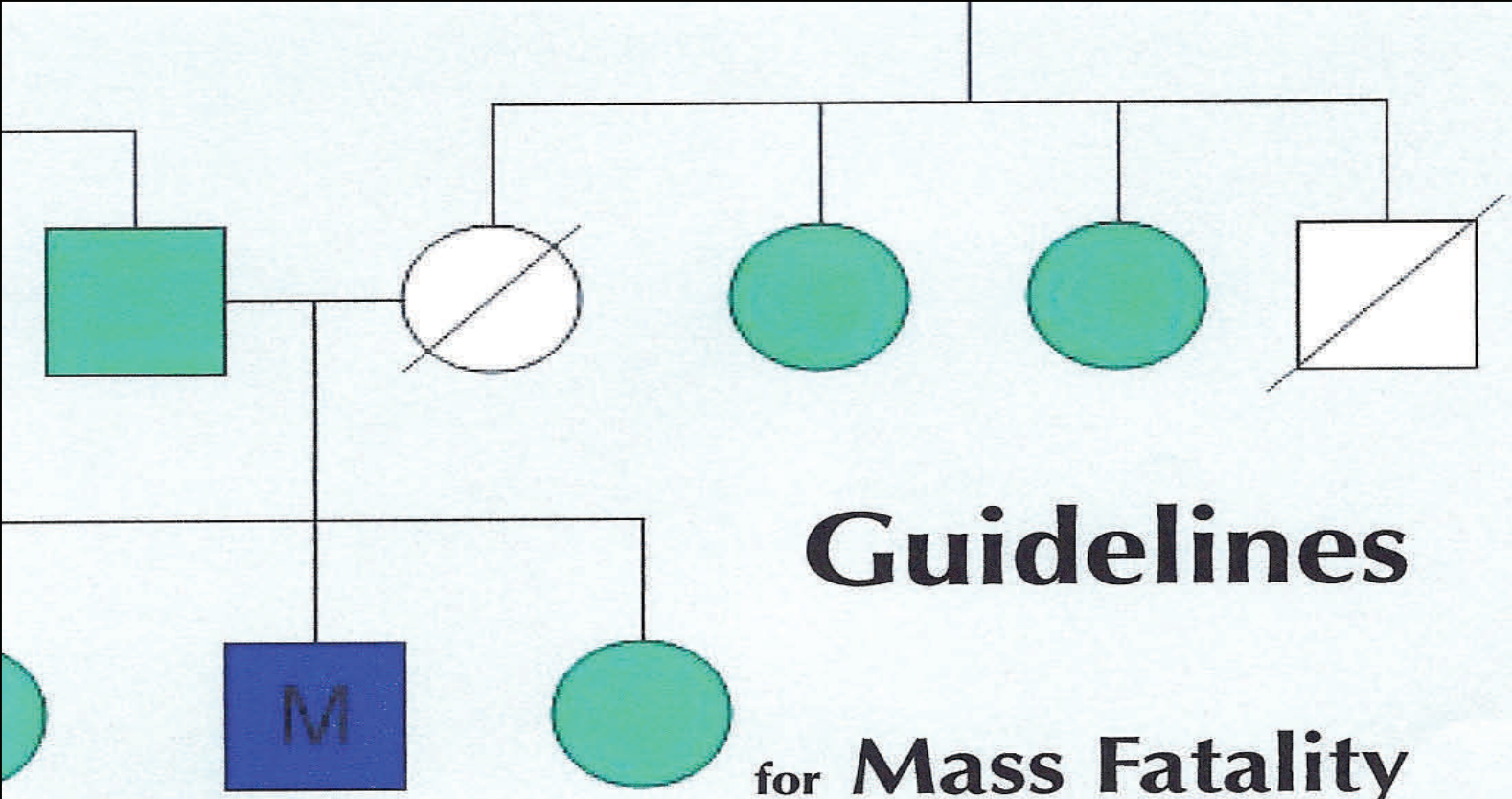

Guidelines for Mass Fatality DNA Identification Operations

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Advancing Transfusion and Cellular Therapies Worldwide

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Efforts are made to have publications of the AABB consistent with regard to acceptable practices. However, for several reasons, they may not be. First, as new developments in the practice of medicine occur, changes may be recommended to the *Standards for Relationship Testing Laboratories* and *Standards for Blood Banks and Transfusion Services*. It may not be possible, however, to revise each publication at the time such a change is adopted. Thus, it is essential that the most recent edition of the *Standards* be consulted as a reference in regard to current acceptable practices. Second, the views expressed in this publication represent the opinions of the authors. Publication by AABB does not constitute an endorsement by the AABB of any view expressed herein, and the AABB expressly disclaims liability arising from any inaccuracy or misstatement.

Contents

PREFACE

These *Guidelines for Mass Fatality DNA Identification Operations* were prepared by a working group of subject matter experts for the AABB Relationship Testing Standards Program Unit with input from other members of relevant disciplines and organizations. The goal of this working group was to present an overview of the different factors involved in a mass fatality DNA identification operational response. This is an attempt to comprehensively address all of the many complex issues together to provide guidance to those involved in such a situation.

This document is designed to be overarching and applicable to decision makers as well as laboratory managers and scientists in planning and implementing mass fatality response DNA identification operations. It must be emphasized that this document provides guidance, and does not set standards. The guidance is based on lessons learned from previous mass fatality DNA identification operations.

We appreciate the contributions of the authors and the thoughtful input from numerous reviewers. Readers are encouraged to submit constructive comments for planned updates of the document by sending feedback to publications@aabb.org.

George Riley, PhD
Amanda Sozer, PhD

INTRODUCTION

A range of incidents—from air traffic accidents to armed conflicts to natural disasters—can cause mass fatalities. Following a mass fatality event, human remains are identified for the purpose of repatriation, issuing legal documents, and certifying death. DNA operations support this effort by issuing relationship and/or match reports. These reports are incorporated along with non-DNA evidence by the entity responsible for making the final identification determination of the human remains.

These *Guidelines* provide support for the development of efficient and effective mass fatality DNA operations. The topics covered include program management and oversight, technical considerations in sample collection and testing, data review, screening and statistics, and reporting. Special considerations addressed include family interactions, educational and psychosocial support, and confidentiality of data.

Several appendices are included to assist in calculating kinship, determining probabilities, addressing mutations, handling pedigree discrepancies, and using family pedigrees for optimal sample collection. Key references are provided where available.

Readers will notice that some information is repeated for emphasis in multiple sections. This is intentional, to reinforce critical concepts for all audiences, including high-level government officials and those with technical expertise who manage or support technical operations.

I. PROGRAM MANAGEMENT AND OVERSIGHT

Because the success of the DNA effort is tied directly to the management of the DNA program, appropriate management should be defined before beginning the DNA effort. The decision to use DNA analysis to identify human remains should be predicated on the scope of work and whether it is possible to achieve meaningful results. Factors that affect the complexity of the DNA identification effort include:

- Number of individuals reported missing.
- Number of individuals presumed missing.
- “Open” vs “closed” list of victims.
- Number of victims.
- Condition of human remains.
- Degree of disarticulation.
- Commingling of remains.
- Degree of degradation.
- Availability of reference samples.
- Funding.

A. General Considerations

Before the DNA identification effort begins, consideration should be given to the following:

1. DNA typing can be expensive. Calculation of an adequate budget and identification of appropriate financial resources are critical to success.

2. Reference samples should be available to identify the human remains.

3. Appropriately trained personnel, equipment, reagents, and supplies should be available to support the entire operation. If outside resources are used to support different aspects of the identification effort, the appropriate contracts and/or written agreements such as memoranda of understanding should be in place.

4. Information technology infrastructure will be needed to support the data associated with a DNA identification effort.

5. Informative profiles from human remains are essential for a successful identification operation. When the ability to obtain informative profiles from human remains is in question, viability studies should be conducted before beginning human identification efforts to evaluate the success rate at which informative DNA profiles can be generated from the human remains.

6. The overall cost and the benefit to the community should be carefully evaluated and communicated before commencing DNA identifications. It is important to avoid establishing unrealistic expectations regarding the outcome of the effort.

7. The response time to a mass fatality incident plays an important role in the overall organization of a DNA identification operation and can directly impact the ability to provide useful DNA information. Longer response times may increase the likelihood of complications during the DNA identification operation, as well as the financial resources necessary for the successful completion of DNA identifications. Response time is dependent on existing infrastructure, such as existing mass disaster plans, available financial resources, existing scientific capacity, and access to DNA laboratories. Response time can also be dependent on the implementation of the necessary legislative changes to provide a legal context and basis for supporting DNA human identifications and victims' families according to accepted international practice.

8. DNA analysis is one method of scientific human identification and should be performed in coordination with other human identification methods such as anthropology, pathology, dental, and fingerprints. In addition, because DNA analysis is costly and time consuming, it should be carefully considered if other scientific identification methods can provide accurate results without DNA. However, this should be balanced by specific advantages that can be gained by sampling and/or DNA typing even conclusively identified remains (for purposes of re-association, additional family matches, or quality assurance).

B. Laws and Regulations

All aspects of the DNA identification effort should follow applicable laws and regulations.

C. Data Centralization

Screening unknown profiles against reference profiles is the process that generates potential mass fatality DNA matches. Although testing can take place in a number of different locations, it is important to centralize the data analysis to ensure uniformity and quality so that potential matches are not missed.

D. DNA Identification Project Plan

Before beginning a DNA identification effort, a project plan should be developed or carefully reviewed by individuals with previous mass fatality experience. The plan should address the following:

1. Survivor/Family Right to Privacy and Safety

The definition and execution of the right to privacy in an identification effort can have direct implications on the personal safety of the surviving families. Personal safety in such instances often depends on the political, cultural, social, and religious context in which the surviving family member(s) live. The operating definition of these privacy rights will affect every aspect of the identification effort, beginning with staff selection, collection and management of data and samples, information management (DNA profile database), and reporting of results. Examples of this are:

a. **Socio-cultural considerations:** Social, cultural, and religious customs can be a significant factor in determining how family members of victims of mass fatalities are treated and perceived in their respective societies. For example, data security and privacy are critical; leaked information about discrepancies in social and genetic pedigrees can potentially lead to harmful action taken against the biological mother.

b. **Political considerations:** In contexts where human rights violations are a consideration, the potential exists for collected data and reported results to cause harm. In many countries, the simple act of collaborating in an identification effort of victims of mass fatalities could be viewed by the local governments as threatening, resulting in recriminating actions taken against family members.

c. **Legal considerations:** Legal limits and vacuums might exist in regards to the right to privacy and the resulting potential for harm for surviving family members contributing to identification efforts. It is important to identify and consider these legal issues before implementing an identification operation.

2. Organizational Structure

The organizational structure should facilitate the efficient and effective collection and testing of samples and reporting of results.

a. In order to maintain consistency, quality, and centralization of data, there should be one agency or organization that leads the DNA identification effort.

b. An organizational chart or equivalent document should be developed. All participants should have clear roles and responsibilities and follow written procedures.

c. The organizational structure should support effective communications between the different components of the operations (ie, between sample collection and data analysis).

3. Fiscal Responsibility

DNA identification efforts are complex. Sources of funding should be identified before commencing the operations. A budget should be developed that encompasses all aspects of the operations, taking into account the regulations on spending various funds.

4. Concluding the Operations

The plan for concluding the operations should manage family expectations, maximize fiscal resources, and address possible identifications after conclusion of the current operations. The disposition of samples and data should also be addressed.

5. Testing Strategy

It is important to define a testing strategy that will maximize identifications and minimize cost.

a. Standard and well-established methods should be used whenever possible. Experimental technologies should be used only after careful consideration and thorough evaluation. Independent review groups can be helpful in evaluating the applicability of the new technologies.

b. Laboratory experience and capacity should be carefully evaluated. Control samples and sample failure should be incorporated in estimates of the volume and cost of testing. If the laboratory does not have the capacity to handle the testing, outsourcing should be considered.

c. Introducing standard and well-established technology and equipment that is new to the laboratory for the purpose of the mass fatality operation should be carefully evaluated before implementation. Considerations include validation, implementation, training of staff, and failure rate. Implementation difficulties with new technologies can waste precious samples, time, and resources.

d. The laboratory(ies) performing the testing should have proven experience in processing the sample types and required throughputs. Multiple laboratories can be considered to meet the needs for processing sample types and throughput.

e. Careful consideration should be given to the choice of testing methods to ensure the efficient generation of useful data and meaningful results. If multiple laboratories will be performing testing, the DNA loci tested must be compatible and as consistent as possible to maximize results, especially when kinship analysis is performed. If laboratories use different loci, data cannot be compared.

6. Personnel

A mass fatality response operation may require a surge in staffing. There should be a mechanism in place to ensure there isan adequate number of qualified individuals to support the operations.

a. All personnel (including volunteers and contractors) should be qualified to perform their assigned tasks. Training and competency assessment should be employed and documented. Performance expectations should be clearly defined through the use of job descriptions, or in the case of consultants and volunteers, through contracts or other written agreements.

b. Because DNA operations require working closely with the families to communicate the process, and to obtain required information, it is easy for the staff involved in a mass fatality response to become close with the family

members. In order to maximize objectivity, the analysts who are interpreting the data and issuing the reports should not interact with the families.

7. Psychosocial Support

Identifying human remains is a complex and stressful process and can take a psychological toll on both the families and the staff supporting the families and identifying the remains. Appropriate psychological support should be available to the staff to minimize the potential for trauma.

8. Generating a Missing Person's Case

A DNA case will need to be created for each possible reported missing (RM) person.

a. All available reference samples should be documented in this case.

b. A unique number should be assigned for each RM. Depending on the type of mass fatality (eg, open vs closed, extremely large number of reported missing persons, mass fatalities that cross multiple jurisdictions, etc), it may require considerable resources to ensure that a unique case number is assigned for each possible missing person.

c. In a mass fatality with an open system (where the names and/or the numbers of deceased are not certain) individuals initially reported missing may not actually be deceased. Reference samples may be collected from families, but before DNA testing of collected reference samples begins, it is important to verify that the RM has not been found alive. In a large incident this may be complex and require significant resources. It is an ineffective use of resources to perform testing on reference samples for individuals who have not actually perished in the mass fatality.

9. Interaction with Family

The family plays a critical role in assisting the DNA laboratory by identifying appropriate reference samples and helping obtain reference samples for the missing person. Families are distraught and need to be supported in order to obtain as much information as possible.

a. It is important to provide the families with clear instructions and information about the sample collection and testing process.

b. Clear communications and accurate information will help manage expectations and ensure cooperation throughout the lengthy and complex process.

c. Providing families with information about other agencies and resources can support them as they cope with stress and grief.

10. Sample Collection

The agency responsible for the DNA operations should begin immediately to implement standard collection kits and documentation, and establish training for individuals designated to be involved in the collection process.

a. Policies and procedures for scheduling, collecting, and transporting samples to the laboratory should be implemented.

b. Adequate resources should be allocated to appropriately collect samples to maintain integrity and chain of custody. Family members may be located around the world.

c. Often people and well-meaning agencies will collect their own samples and send them to a DNA laboratory. Mislabeled samples or inconsistent sample quantity and quality will result in confusion and additional work for the DNA operations.

11. Sample Identification and Chain of Custody

The collection and testing procedures should include a numbering/identification system for uniquely identifying each sample and its derivative(s).

a. This system allows samples and derivatives to be connected with their associated information, such as individual's name, collection site, collection location for human remains, collection date, etc.

b. Names or descriptions are not sufficient to uniquely identify samples.

c. It may be very helpful to those performing data review to have a numbering system that indicates the sample type and testing laboratory in the number.

d. Sample collection, handling, storage, and documentation procedures need to be established to maintain chain of custody.

12. Sample and Information Tracking

Accurate and efficient tracking is critical to efficient testing and workflow. This is especially important if multiple testing laboratories are used to generate the DNA profiles.

a. It is important that the DNA operations have a system in place to maintain and track cases, samples, and data.

b. Laboratory information management systems can be very helpful to track samples and generate work lists that identify what work is required to be performed on each case, sample, data set, or profile.

13. Data Integrity

The success of the DNA identification operations depends on accurate data.

a. Control of data collection, entry, access, and storage following standardized procedures is critical to data accuracy.

b. Appropriate resources should be allocated to maintaining data integrity.

c. Independent verification of data entry into electronic format should be performed in order to ensure data quality and accuracy.

d. Manual data entry should be minimized.

e. Electronic transfer of data should be validated.

f. Data management and flow should allow for transparency, independent verification, and review of the conclusions if questions arise involving the testing and data interpretation.

g. Data storage and access should be controlled by one central location/entity.

h. Data storage should be maintained and secured to ensure integrity and prevent loss. Data backup should take place regularly and should be verified.

14. Quality Program

A comprehensive quality program is critical to ensuring efficient and accurate testing and reporting.

a. Testing standards should be reviewed and followed to the extent applicable and possible. The references and suggested readings included as part of this document should be reviewed before operations begin.

b. Periodic internal and external documented assessments can be helpful in assessing and improving quality and testing operations.

15. Reporting

Records substantiating conclusions should be maintained. The case file and associated records should contain sufficient information to allow an independent analyst to reach the same conclusion that was contained in the report. The DNA operation should have a policy on who receives the DNA reports, and what information is included on the reports.

16. Sample and Record Storage

Samples and records should be maintained in a manner that will maintain integrity and minimize deleterious change. Storing records and samples in an organized manner will facilitate operations, independent review, and possible project hand-off at the conclusion of the operations.

17. Document Control

Rapidly implemented DNA operations for a mass fatality response create challenges for document control. It is critical to document policy and procedure changes. Version control will help ensure that the current policies, procedures, and forms are used. Document control should be simple and flexible to allow rapid and accurate implementation of changes.

18. Public Relations

A policy should be established for communicating with the media and public so that consistent and accurate information is always provided. If multiple laboratories are involved in the testing process it is wise to have all press releases and public statements reviewed and approved by the managing agency prior to being issued.

19. Confidentiality Policy

DNA operations should implement and adhere to a confidentiality policy that protects the privacy of those tested.

20. Safety and Security

The DNA operations should have adequate policies and procedures in place to ensure a safe and secure working environment.

21. Contract Management

Carefully considered contracts and agreements with other agencies and organizations will support the effective use of outside resources. The following should be considered when entering into contracts:

a. Identification of needs and requirements of the identification effort.
    - Deliverables and due dates.
    - Roles and responsibilities.
    - Quality metrics.
    - Confidentiality.
    - Use of data.

b. Procurement.

c. Contract management and possible modifications.

d. Fiscal responsibility.

22. Offers of Support

Companies, organizations, and individuals interested in helping with the identification effort may provide offers of support. Careful consideration should be given to any donation.

a. The DNA operation should independently identify and specify its needs before accepting donations.

b. Written agreements (eg, contracts and memoranda of understanding) should be used whenever possible.

c. Novel and/or outdated technology, equipment, and supply donations that do not meet the needs and requirements of the operation should not be accepted. Integration of equipment or supplies that do not meet the needs of the operation is an inefficient use of valuable resources. One of the most valuable resources in an operation is the time of the skilled workers.

d. Donations should be evaluated and thoroughly validated as if they were purchases.

II. TECHNICAL CONSIDERATIONS - SAMPLE COLLECTION

A. Human Remains

Sample selection should target the biological specimen with the highest potential for successful DNA extraction and typing. Remains should be evaluated and, depending on the environmental insults that may have occurred, a secondary sample type might need to be collected as a potential backup.

1. ***Duplicate Samples***—Whenever possible, duplicate samples should be collected. Duplicate packaging should be evaluated for efficiency in collection, ease of sample identification, and accuracy in maintaining the chain of custody. Each duplicate sample should be assigned a unique identifier when packaged separately.

2. ***Laboratory Capabilities***—The testing laboratory should be consulted for capabilities and efficiency in sample processing. Often it is easier and faster for the laboratory to process a particular type of sample. The laboratory processing the samples should have proven capabilities with regard to sample type and throughput. Laboratories with insufficient or uncertain throughput capacity or experience with similar samples should consider outsourcing the testing.

3. ***Sampling***—Unless it has been carefully considered and it will not disrupt the overall identification effort, collection should take place only after the other postmortem evaluations (dental examination, anthropological analysis, etc) have been completed.

4. ***Sample Types***—Laboratories may have different minimum sample requirements. The types of samples and sample amounts need to be defined by the laboratory(ies) performing the testing. The following are examples of specimens that may be requested by the laboratory.:

    - Blood or blood clot from fresh remains.*
    - Skeletal muscle.
    - Natural nails.
    - Long bone (avoid freshly broken ends and anthropological landmarks).
    - Teeth with crown and roots without restorations.
    - Other soft tissue* or bone specimens.
        - Samples such as skin and vertebrae should be avoided where possible because they may require additional resources to generate a profile.
    - Severely burned remains.
        - Valuable information can be obtained from burned (not calcined) bones.
        - Teeth also serve as a good resource due to the protective enamel.

    *Note: Soft tissue is not likely to give results in cases where the remains are subject to degradation or putrefaction.

5. ***Fragmented Remains***—It is important for the DNA identification operation to coordinate with the entity responsible for making the final identification determination of the human remains and/or the relevant authority associated with the mass fatality identifications on how to handle fragmented remains.

    a. When fragmented remains are collected, it is necessary to determine whether all remains, only recognizable remains, or only remains of a certain size will be tested.

    b. Samples collected from individual fragmented and disassociated remains should be uniquely identified separately, even when they are thought to come from the same individual. Multiple samples collected from a single contiguous remains fragment may be given unique identifiers showing that the remains were subdivided. Samples taken from noncontiguous remains (ie, more than one piece of remains) should be given different unique identifiers because remains from different individuals may be inadvertently packaged together.

6. ***Identified Remains***—Specimens should also be taken from remains that have been positively identified by other conventional methods. The results from these specimens may later serve as reference profiles for fragment re-association or as family references for other identifications.

7. ***Sample Consumption***—If it is deemed necessary to consume the entire sample for DNA analysis, the entity responsible for making the final identification and/or the relevant authority associated with the mass fatality identifications should be informed that the entire sample may be consumed in testing. This situation may lead to a positive identification with no remains available to return to the family.

8. ***Collection and Handling of Samples***

   a. Samples should be collected to minimize the risk of loss, contamination, or deleterious change.

   b. It is important to place samples in individual leak-proof containers and appropriately label them with a unique identifier using permanent markers or computer-generated labels.

   c. Samples should be stored in a secure area under conditions that minimize degradation.

B. Reference Samples

1. ***Reported Missing***—A Family Assistance Center (FAC) should be established as soon as possible to identify individuals who are truly missing.
   a. A list of missing persons (victims) should be generated and a method for avoiding duplicate listings of reported missing persons (RM) should be implemented. Each missing person should be assigned a unique identifier for information management purposes.
   b. Family pedigrees should be established for each missing person as information is gathered from family members.

   c. While family members are at the FAC, it is recommended that reference samples from as many appropriate family members as possible be collected for each victim. See Appendix F. It is better to have samples from more family members than required than to go back at a later date and collect from those family members. The laboratory may use the family pedigrees to determine which samples will provide the most useful genetic information for identification purposes.

2. ***Collection Plan***—A plan should be developed to collect reference samples (family references, direct references, and/or personal items) at the FAC as well as remotely. The plan may include the use of contracted collectors. A location should be established for receipt of reference samples.

3. ***Sample Types***—The laboratory should be consulted on the best samples to collect. Common reference sample types include:

   a. Family References – It is important to understand the family members collected will be unique for each family based on the family structure and the availability of individuals to provide samples. The key concept is to start with the RM on the family pedigree, and follow all of the lines along the pedigree to each of the RM's biological relatives without skipping a person who is able to provide a sample. Samples from individuals beyond those who are available will not provide any additional genetic information in terms of DNA identification. See Appendix F.

      - Whole blood collected in tubes with preservative (EDTA or ACD tubes) or dried bloodstains from venipuncture or fingerstick.
      - Three to four buccal swabs. The swabs should be air dried in a manner that precludes sample mix-up and placed into a breathable envelope (ie, paper).

   b. Direct References – Biological samples documented to have come from the missing person before death are extremely useful reference samples. These direct reference samples were typically collected by a medical professional, such as a doctor or nurse during a medical procedure or test. Samples of this nature can be directly attributed to the missing individual through the associated medical record. The sample's provenance and link to the missing individual should be documented. The DNA laboratory should discuss the potential availability of these samples with the family and then follow up with the establishment that might possess the sample. Common types of direct references include:

      - Blood samples or bloodstains (from DNA repositories or hospitals).
      - Pathology specimens (biopsy specimens, Pap smears).

c. Personal Items – Personal items used by the missing person can be effective in identification. However, it is important that the item be definitely identified as belonging to the missing person by someone with direct knowledge, and that the item have been used only by the missing person. When possible, it is best to collect at least two personal items and also family references to allow validation of the personal item. Some examples of personal items include:

   - Toothbrush.
   - Unlaundered clothing.
   - Used razor.
   - Nail clippers.
   - Hair brush (hair).
   - Stamps or envelopes licked by the individual.

d. Elimination Samples – Elimination samples are biological samples taken from individuals who could have potentially introduced their DNA into the testing process (for example, by sharing the RM's personal item). These samples are processed by the laboratory to generate DNA profiles, which are then compared for quality assurance purposes and to eliminate them as potential DNA contributors to personal items. Elimination samples should be requested from anyone (including DNA staff) who could have potentially introduced their DNA into the testing process.

C. Collection Procedures

Collection and sampling procedures should be established in protocols that address:

1. Human Remains

a. All remains should be described, inventoried, and photographed as applicable before sampling.

b. It is important that each sample be uniquely identified; a name is not an identifier. The unique identifier should ensure traceability of the remains, allowing it to be connected with its associated information such as collection site, date, etc.

c. It is important to initiate and maintain a Chain of Custody (CoC) utilizing unique identifiers. Included should be an identification of the individual who performed the collection; the location, time, and date the sample was collected; the sample origin; and a description of the specimen collected.

d. It is critical for CoC documents and other paperwork accompanying human remains to be written in waterproof ink and be packaged in a separate sealed plastic bag to prevent loss of the information during shipment or temporary storage.

2. Reference Samples

a. Donor identification and consent forms.

b. Documented specific relationship to victim (eg, full sibling vs half sibling).

c. Proof of identification (eg, government-issued identification, fingerprint, or witness testimony).

d. Purpose for requesting sample and intended use.

e. The Donor should sign a form to authorize sample use and testing. A policy for authorization should be in place for samples collected from individuals who cannot read and write or who require interpretation.

f. Sample packaging should be sealed and labeled with a permanent label.

g. Sample packaging may be signed or initialed by the donor to verify the accuracy of the label.

h. The CoC should be initiated and maintained and should include the donor's name; the individual collecting the reference; and the location, time, and date of collection.

i. Notification to the family that a personal item or direct reference item could potentially be destroyed in the testing process.

III. TECHNICAL CONSIDERATIONS - TESTING

A. Sample Handling and Storage

1. An inventory system should be developed to log and track samples.

2. Each specimen should contain a unique identifier. If multiple laboratories are processing samples, the laboratories should use the same consistent numbering system. If barcodes are used, it is important that appropriate equipment and software are available at each laboratory to read and print the barcode format and identifier.

3. Specimens should be stored to prevent contamination and degradation. Consideration should be given to the anticipated volume of samples and the short-term and long-term storage goals.

B. Extraction

1. It is important to use extraction methods and protocols that are appropriate to the sample type and condition to ensure the highest possible success in recovery of usable DNA.

2. High-throughput processing (eg, robotics or batch processing) can be advantageous for mass disasters involving a large number of individuals and/or incidents with a high degree of fragmentation. Many robotic platforms are also available for processing blood and/or buccal swab references. However, if robotics are not already used in a laboratory, it may be difficult to validate and implement such processing in response to a mass fatality event.

3. Laboratories that are significantly increasing their processing throughput or batch size should validate the entire extraction process before implementation of testing. The batch sizes used during validation should be similar to those planned for the disaster sample and reference sample testing.

4. It is important for the laboratory to have experience with poor quality and low quantity samples when degradation is present or sample size is limited and for the laboratory to demonstrate that its extraction methods do not introduce contamination.

C. Analysis

1. The laboratory should use appropriately validated DNA analysis procedures. If contract laboratories are used to do the testing and analysis, the analysis and data interpretation procedures used in the contracting laboratories should be reviewed and approved by someone with appropriate technical expertise.

2. All laboratories (including contract laboratories) should have and follow a quality system. Ideally, laboratories should be accredited.

3. Before generating any data, it is important for the managing agency to determine the genetic systems and the set of loci for comparison. This is best accomplished by collaboration with the testing laboratories to ensure that effective and compatible loci are tested.

4. The laboratory should devise a plan for incorporating multiple genetic systems if needed (eg, when there is no close relative or there are insufficient corresponding personal items or remains specimens for the victim). Mitochondrial DNA, Y chromosome DNA, and X chromosome DNA testing can be effective tools in these situations. When mitochondrial DNA, Y chromosome, and/or X chromosome DNA testing is performed, laboratories should be employed that have significant forensic experience with these methods. Mitochondrial DNA testing in particular poses technical challenges. If these alternate genetic systems are used, procedures for statistical interpretation should be followed (based on appropriate population databases), as well as procedures for combination with statistics from other genetic systems.

5. All allele calls (allele assignments) and corresponding matches should be reviewed and documented. Review procedures should include guidelines for interpreting partial profiles and creating composite profiles.

D. Data Management

1. It can be advantageous for the DNA operations to have access to a central database containing data from antemortem interviews and all postmortem information (eg, fingerprints, pathology reports, dental records, etc) to aid in coordinating efforts in the DNA identification process.

2. A system should be established for tracking paper files.

3. Appropriate software should be used for storing and searching DNA profiles from victims and references. It is important to use software that is capable of calculating complex family reconstructions and doing familial searches if any family references will be used.

4. If multiple laboratories are performing the analysis, the laboratories should decide on the appropriate data format for transmitting data. A single laboratory should be identified for interpreting results and performing searches.

E. Sample Disposition

1. The entity responsible for making the final identification determination of the human remains (Medical Examiner/Coroner) should establish a plan with the DNA operations with regard to disposition of unknown and reference samples once analysis has been completed.

2. Because advancements in science may allow more effective testing, sample retention and preservation may be considered for future testing. In that case, special attention should be given to the ethical issues addressed in Section VII.

IV. DATA REVIEW

A. Acceptable Controls

All positive and negative controls (amplification and reagent) should meet predefined criteria.

B. Profile Quality

1. To ensure data quality and profile accuracy, all DNA profiles used in the process of making a DNA identification should meet defined criteria with respect to peak morphology, peak height, peak height ratios, dropout (one or both alleles not detectable), artifact determination (stutter, spectral artifacts, etc), allele and locus nomenclature, internal ladder standards, allelic ladders, contamination, and acceptable amplification controls.

2. Typically, quality criteria are determined through validation studies. Information on validation studies can be found in the Revised SWGDAM Validation Guidelines published in *Forensic Science Communications*, July 2004 (http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_ 03_standards02.htm) and also on the US National Institute of Standards and Technology Web site (http://www.cstl.nist.gov/strbase/vali dation.htm). In addition, data quality parameters may be set so that data can be reviewed easily and rapidly before screening and interpretations take place.

C. Sample Validations

Profiles should be evaluated to verify their accuracy.

1. Profile Comparisons

To detect and eliminate errors caused by accidental switching of samples or testing anomalies, two independent DNA typings of each sample are recommended. Verification that the DNA profile from the sample is the same every time it is tested guards against sample switches and other potential problems with quality assurance. Data management software can automate this task. Alternatively, a DNA profile may be verified by comparing it to known family reference samples to verify the samples' relationship within a family.

2. Gender Verification

Using the DNA marker amelogenin, the gender of reference samples should be verified against the gender indicated at the time of sample collection. The gender of the remains samples should be compared whenever possible to the gender identified in the postmortem report and any discrepancies investigated and resolved. Y-chromosome STR analysis can be used in the event of suspected null alleles.

3. Direct References and Personal Item Validation

a. Profiles from personal items and direct references should be compared to the putative family reference samples and evaluated for kinship. Kinship calculations should support the reported social pedigree.

b. Profiles from personal items and direct references should be compared to RM-specific elimination samples. Personal item samples that produce mixtures should not be used unless an elimination sample allows subtraction of sufficient alleles and/or genotypes from the mixture to produce the DNA profile of the RM person.

4. Kinship Sample Validation

a. Kinship calculations of family reference samples should be performed to ensure that the reported social pedigree and biological kinship determinations agree. When discrepancies between social pedigrees and biological kinship are detected, they should be investigated. Careful elicitation of additional information from the families by qualified and trained persons may resolve these discrepancies. Testing using additional systems (Y-chromosome STR, mitochondrial DNA, etc), samples from additional family members and/or direct references or personal items

belonging to the missing person may be required to resolve the discrepancy. See Appendix D for a discussion of genetic mutations in kinship analysis.

b. The ensemble of reference profiles typed for RM cases can be checked in advance to determine whether the profile ensemble is sufficient to match to the respective human remains. Kinship software can simulate possible DNA profiles for the RM person and calculate the relationship indices for each simulated profile. If the simulation indicates that the profile ensemble is insufficient, additional reference samples should be obtained.

V. SCREENING AND STATISTICS

A. Population Databases

1. It is important to select the proper population database. An incorrect population database can distort the significance of allele sharing because common alleles in one population database may be rare in another. Population databases for many ethnic groups are published in journals and are available for public use. Creation, validation, and use of a novel population database may be advisable when focusing on small or unique populations that may not be well represented by published databases.

2. For screening, relevant population databases from the representative ethnic groups of the missing population(s) should be used. If technology limitations allow screening in only one population database, the predominant ethnic group of the missing persons should be used.

3. Results should be reported for all relevant population databases or the database with the lowest statistical value. Calculations should be performed in all relevant population databases and the results from each should meet the match threshold criteria.

4. When the missing person is of known mixed ancestry, the combined relationship index (CRI) or combined direct match statistic should be calculated locus by locus in all of the relevant population databases. For each locus, the population database result with the highest frequency or lowest discriminating power should be used in calculating the CRI or combined direct match statistic.

B. Screening

1. Screening is the process of comparing DNA profiles from remains against DNA profiles from reference samples to detect possible matches. Screening should take into account genetic inheritance and allele frequency to help eliminate spurious matches and identify kinship even when few alleles are shared.

a. Statistical methods calculate a likelihood ratio (LR), called a relationship index, for each remains sample against each family reference sample. Relationship indexes can be grouped by family to give a combined score. Because this method is complex, only those software packages that are carefully designed and validated for this purpose should be used.

b. *Allele sharing methods, which count the number of alleles shared between a remains sample and a reference sample, do not calculate the statistical significance of the match. These methods, therefore, do not indicate how likely the match is to occur between two unrelated individuals. This method should be avoided whenever possible.* Although this method may appear preferable, it produces many spurious matches that are time-consuming to investigate and may miss kinship matches that are based on a small number of shared rare alleles.

2. Once kinship analysis verifies that a direct reference or personal item is attributable to an RM, the personal item profile can be used to preferentially screen for potential matches.

C. Direct Matches

1. When available, direct references are preferable because they avoid the complications that can be observed in kinship testing (mutations, non-relatedness, etc) and provide more powerful statistical information. Laboratories may wish to consider developing a policy for reporting direct matches if profiles match at a predetermined statistical weight or number of matching loci with no inconsistencies.

2. Appropriate statistical calculations should be used. National Research Council (NRC) II recommendation 4.1 (The Evaluation of Forensic DNA Evidence, 1996) provides guidance on calculating random match probabilities. Accepted equations for statistical calculations can be found in NRC II Equation 4.4a for homozygotes and 4.1b for heterozygotes.

When a match has been identified, an estimate of the probability of the match having occurred by coincidence can be determined. See Appendix A for additional explanations on performing random match probabilities.

3. Samples from human remains or personal items may produce incomplete profiles. All loci should be evaluated for consistency between the profiles even if the locus is of insufficient quality to be included in the statistical calculation or reported.

4. Loci with no useful information can be excluded from the calculation. Loci with partial information (eg, uncertain zygosity or possible allele dropout) should be carefully evaluated and it is important that they not be ignored if they are not consistent with the overall match. A good example is a remains sample that produces an allele of 13 at one locus. Based on the quality control criteria, it is unclear if the type is 13,13 or 13,?. This means that a 13,? could potentially match a 13,17, but a 13,? would not match a 14,15 and should be investigated. See Appendix A for a statistical discussion about partial profiles.

5. When the quality of both the reference sample profile and the remains sample profile are compromised, it is important to compare the profile locus by locus to ensure that there are no discrepancies or inconsistencies. This is mainly a problem when remains samples are compared to personal items.

D. Kinship Calculations

The statistics used in kinship-based DNA identifications require a formalized consideration of investigative information, the DNA profiles obtained, and the reported genetic relationships between families and missing persons (ie, the pedigree). A Bayesian approach as discussed in Appendix B is recommended for this analysis.

1. Prior Probability

a. Investigative information such as the number of missing persons is incorporated into the calculation in a form known as prior probability. Prior probability is typically expressed as 1/number of missing persons. Therefore, as the number of potential victims of a mass fatality rises, the prior probability of a correct identification decreases and more genetic information is needed to reach a specified probability of relationship.

b. Appropriate values should be used for the prior probability. The prior probability should be based on the number of missing persons, gender, or other available information associated with the mass fatality. Prior probability should be based on objective and numerical data. Appendix C provides a more detailed discussion on prior probability.

c. As the human remains or victims are accurately identified, prior probability can be modified as necessary throughout the identification effort. In complex cases, prior probabilities associated with a variety of scenarios may need to be considered (eg, considering the number of sibling pairs that exist within the population of the missing).

2. Likelihood Ratio (LR)

a. In a Bayesian analysis, the LR is the factor by which the DNA evidence increases or reduces support to a hypothesis of relatedness. In routine relationship testing, the LR compares (X/Y), where:

> X = The probability of obtaining the DNA profiles assuming the remains are related to the family references in the way described by the pedigree.
>
> Y = The probability of obtaining the DNA profiles assuming the remains are from an individual unrelated to the references, but the references are otherwise related in the way described by the pedigree.

b. The LR is also called the relationship index. Each independent locus tested produces its own relationship index, which can be multiplied by those of other independent loci to calculate a combined relationship index (CRI). If genetically linked loci are used, then haplotype frequencies should be employed. See Appendix B for a further discussion of calculating likelihood ratios.

c. The entire set of validated kinship reference profiles should be used in a single calculation to produce the LR. For example, if the reference profiles include two siblings, a parent, and a child, they should all be compared in a single calculation rather than independently or separately in smaller groups. Because this method is complex, only those software packages designed and validated for this purpose should be used. Individual comparisons may result in false

identifications, especially when there are related victims. In addition, the individual comparisons do not yield the same level of statistical significance as a comparison involving the entire reference group.

d. Kinship analysis involving related victims is complex. The LR calculations should use all appropriate data from related victims and family members. All possible relationship scenarios should be evaluated. The DNA analyst should be aware of the kinship software capabilities and limitations in relation to complex cases before reporting results. The DNA analyst should be able to communicate the complexities in difficult-to-resolve cases, so that review boards or identification officials can best reach conclusions in light of all evidence in the case.

3. Posterior Probability

The prior probability and CRI are combined to compute a posterior probability of relationship. A discussion of the prior probability, LR, CRI, and posterior probability is contained in Appendix B.

E. Mutations

1. A method for incorporating genetic inconsistencies into the statistical analysis should be decided upon and well understood before testing begins so that mutations are reported in a consistent manner. By choosing the methods in advance, the DNA operations will minimize potential accusations of biased interpretations. A complete description of available mutation calculation methods for paternity and maternity cases can be found in Appendix D.

2. Regardless of the method chosen, the mutation should be included in the CRI. See Appendix D for a discussion of mutations.

3. The publication(s) used for determining mutation rates in kinship calculations should be documented.

4. In mass fatality operations that use kinship analysis, mutations will be routinely encountered due to the large number of meioses and the large number of locations that are tested.

F. Null Alleles and Incomplete Profiles

1. Sequence variation in primer binding sites may cause the apparent loss of an allele. Null alleles should be dealt with appropriately, such as by amplification of the same locus with alternative primer sequences and/or treating the locus as a mutation.

2. Samples from human remains may produce incomplete profiles. All loci should be evaluated for consistency between the profiles and the reference profiles of the provided family pedigree, even if the locus is of insufficient quality to be included in the statistical calculation or reported.

G. Differences between Social and Genetic Pedigrees

1. DNA testing may uncover situations where individuals are not related in the manner in which they described their relationship. The DNA operation will need to carefully consider how this information will be handled, taking into account legal considerations and the impact on families.

2. During the course of kinship testing, it may become necessary to modify the provided social pedigree to what is believed to be the biological pedigree based on the genetic testing. If the family cannot provide helpful information, deductive reasoning should be used to make educated alternative hypotheses. It is important to take into consideration all possible causes for the inconsistency in the social pedigree, including additional related missing persons or any other combination or possible explanation for the obtained DNA profiles. Sometimes it is not possible to understand the exact inconsistency in the family but use of all available DNA testing methods and collecting additional relatives may provide additional information.

3. When a modified pedigree is used to produce a DNA match report, it is extremely important to make sure that the entity that is using the report to make the final identification understands the modifications that were made and any other possible alternative explanations, and adheres to appropriate policies for genetic data protection to avoid additional harm to families. See Appendix E for further discussion of this topic.

VI. REPORTING

DNA match reports should be provided to the entity responsible for making the final identification determination.

A. Ethnic Databases

When victims are from different ethnic populations, the results should be reported using the different relevant population databases. The origin of the population database(s) used in the match report should be documented.

B. Reports

1. Before issuing the first report, an acceptable probability of relationship should be chosen to report an "identity" match report. This probability takes into account what percentage of a random genetic match is acceptable, for example, a probability of relationship of 99.9% assumes that there is a 0.1% probability of a potential random match. Historically, these important policy decisions have been made by panels of individuals with expertise in statistics, genetics, and mass fatalities.

2. When it becomes necessary to issue a report that does not meet the reporting "identity" match threshold, there should be a policy in place that clearly defines the limits of the testing and the weight of the evidence in the report.

3. The kinship report should at a minimum include:

- The individuals used in the calculation and their relationship to the missing person.
- Unique identifier of the human remains.
- Database(s) used in the calculations.
- The combined likelihood ratio(s) obtained.
- Prior probability used in the calculations.
- Probability of kinship for each ethnic group calculated.
- Signature of the person responsible for issuing the match report.

4. The individual making the final identification of the human remains should be made aware of any information regarding assumptions, other related individuals not being excluded, discrepancies (mutations, incorrect social pedigrees, or other abnormalities), and any other relevant information uncovered by the DNA testing that may affect their identification of the human remains. Depending on the situation, this information may or may not be included in the final written report.

5. The direct match report should at a minimum include:

- The item used for identification.
- Unique identifier of the human remains.
- Database(s) used in the calculations.
- Random match probability for each ethnic group.
- Signature of the person responsible for issuing the match report.

6. Depending on how the report is used and the DNA expertise of the person receiving the report, more information may be required in the written report.

7. Any discrepancy in the testing process discovered after the report has been issued should be addressed and resolved. If the discrepancy does not alter the results and conclusions drawn from the results, but is more of a clerical error, an amended report and/or a quality assurance incident report may be submitted at the discretion of the person in charge of the DNA identification. A factor in this decision will be an effort to satisfy the needs of the entity responsible for making the final identification determination. If the discrepancy alters the results or the conclusions drawn from the results, an amended report should be generated and presented to the entity responsible for making the final identification determination as soon as possible.

VII. SPECIAL CONSIDERATIONS

A. Consent

1. Before the DNA operations begin, there should be a determination on how the sample can be used so that the proper authority can be given by the individuals providing a sample.

2. Families/persons providing biological samples, personal information, and/or personal items should legally agree to the use of such data for the purposes defined by the identification project. This should be done via a signed consent form. If allele frequency tables need to be developed and published for the identification project, the authorization to collect and document such data should be included in the consent form.

3. The laboratory should use the sample only for the purposes authorized by the individual on the consent form. For example, if the individual authorizes the use of the sample only to identify a missing person and to be used in the population database, it is critical that the samples not be used for anything else, without prior authorization from the sample donor.

B. Privacy and Release of Data

1. Appropriate steps should be taken to protect the identity and privacy of the individuals.

2. Before the DNA operations begin, a policy should be developed on what information is released by the DNA operations. This policy should follow applicable laws and regulations.

C. Reporting

1. Before the DNA operations begin, a policy should be developed to decide who is authorized to receive the DNA match results, and what information is included in the report. This policy should follow applicable laws and regulations.

2. The DNA testing may uncover situations where individuals are not related the way they think they are. The DNA operations will need to carefully consider if or how this information will be reported, taking into account legal considerations and the impact on families. The DNA operations will benefit greatly from the assistance of genetic professionals such as those with expertise in molecular genetics, genetic counseling, paternity testing, and mass fatalities. See Appendix E.

D. Education and Psychosocial Support

1. Clear and accurate information should be provided to the families. Policies and public outreach initiatives will help manage expectations and ensure cooperation throughout the lengthy and complex process.

2. It is important to take into account the context in which the DNA operation is functioning, including the religious, social, cultural, and political aspects.

3. Individuals interacting with families throughout the testing process should have appropriate training. This may include DNA testing, genetics, cultural context, and psychosocial support (as appropriate). One person may not be able to answer all the questions that a family has. Printed material about the testing process may be helpful.

4. Genetics professionals, particularly genetic counselors, with training in public health genetics and psychosocial counseling are an important asset to the team in a mass fatality response. These health-care providers work to verify biological relationships, construct pedigrees, and communicate compassionately with relatives on issues related to loss.

E. Disposition of Samples

A policy should be developed to address the disposition of samples and data once the analysis is complete. This policy should follow applicable laws and regulations.

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Appendices

Appendix A. Direct Match Probability

Direct match probability is calculated under the assumptions of Hardy-Weinberg equilibrium.
For heterozygous loci, the frequency of the match in the population is determined using the formula:

$$Frequency\ of\ PQ = 2 \times p \times q = 2pq$$

where p and q are the frequency of each allele in the population.

For homozygous loci, the frequency of the match in the population is determined using the formula $p \times p = p^2$ where p is the frequency of the allele in the population. A correction factor (CF) is often included in homozygous calculations to correct for the possibility of subpopulations. The correction factor formula is:

$$Correction\ Factor = [p(1-p) \times \theta]$$

Theta (θ), an estimate of population subdivision, is often assumed to be 0.01.

$$Frequency\ of\ PP = (p \times p) + correction\ factor = p^2 + [p(1-p) \times 0.01]$$

The combined frequency of matching at multiple loci within a racial group is determined by the mathematical product of the frequency of each locus relevant to the match.

Direct match statistics can also be presented as a likelihood ratio. The numerator assumes that the DNA profile of the remains came from the missing person, and the denominator assumes the DNA profile of the remains came from a randomly selected, unrelated person.

$$Combined\ Frequency = Freq(FGA) \times Freq(TPOX) \times Freq(vWA) \times \ldots$$

$$Likelihood\ Ratio = 1/Combined\ Frequency$$

Example:

Locus	Alleles	Allele 1 Frequency	Allele 2 Frequency	Formula	Locus Frequency
FGA	21, 22	0.173	0.189	2pq	0.065
TPOX	8	0.544		p^2+CF	0.298
D8S1179	13, 14	0.339	0.202	2pq	0.137
vWA	18	0.222		p^2+CF	0.051

$$Combined\ Frequency = 0.065 \times 0.298 \times 0.137 \times 0.051 = 0.00014$$

$$Likelihood\ Ratio = 1/\ 0.00014 = 7143$$

The combined frequency can also be stated as:
Approximately 1 in 7143 individuals of the same ethnic group would be expected to match this profile.

The likelihood ratio is properly stated as:
The DNA profiles of the reference and remains samples are 7143 times more likely to be found if the remains sample came from the missing person than if the remains sample came from a randomly selected, unrelated person.

Formula for a direct match with suspected dropout:

For example, a remains sample produces a low-level partial profile. At one locus, a low-level 13 allele is detected. The zygosity of this allele is uncertain. The true genotype at this locus could be 13,13, or a 13 paired with an undetected allele. While it is tempting to simply exclude this locus from consideration, it must be carefully evaluated for consistency in the overall identification. If a personal item was a 13,17 at the same locus, the personal item and the remains sample might have the same source. If the personal item was a 14,17 at this locus, however, the remains sample and the personal item do not have the same source.

In the example where the personal item is a 13,17, two options are available. The first is to accept that the remains sample and personal item could share the same source, but not compute a statistic for that locus. The second option is to compute a direct match statistic for the 13 allele's homozygote genotype frequency plus the heterozygote genotype frequency, assuming that the undetected allele has the frequency 1-p.

$$\textit{Frequency of P,?} = p^2 + p(1-p)\ \theta + 2p(1-p)$$

Appendix B. Kinship Calculations

Bayes Theorem

Applying Bayes Theorem allows the combination of information from the DNA analysis with non-DNA data. Non-DNA data are quantified into the prior odds (P), while DNA data are quantified into the kinship index. Below is a discussion of how to calculate and combine this data to create a combined probability of relationship.

A. Non-DNA Information

1. Prior Probability

Prior probability (Pr) is the strength of the evidence that the individual is related as specified in the pedigree based only on the non-DNA evidence. Prior probability may be based on the number of missing persons, gender, or other available information and should be a predetermined value for each incident, which may change as identifications are rendered. In complex cases, prior probability associated with a variety of scenarios may need to be considered (eg, considering the number of sibling pairs that exist within the population of missing persons).

2. Prior Odds

Calculation of prior odds is necessary to combine the non-DNA information with the DNA information. Prior odds are calculated using the prior probability as follows:

Prior Odds = Prior Probability/(1-Prior Probability) = Pr/(1-Pr)

Example:

Seven unrelated males are reported missing.

The prior probability of any set of remains being a specific reported missing is = 1/7 = 0.143 or 14.3%

Prior odds = 0.143/(1-0.143) = 0.167

Appendix C provides a more detailed discussion on prior probability and odds.

B. DNA Information

1. Likelihood Ratios

In relationship testing, likelihood ratios are also referred to as kinship indexes or relationship indexes. They are typically used to statistically define the significance of matching DNA loci in relationship testing.

A likelihood ratio (LR) expresses the likelihood of obtaining the DNA profiles under two mutually exclusive hypotheses. The first hypothesis is that the remains sample is related to the family as reported. The second hypothesis is that the remains sample is unrelated to the family, but that the family pedigree is otherwise accurate. The LR in relationship testing is also called the relationship index, and each independent locus tested produces its own relationship index. This index can be multiplied by those of other independent loci to calculate a combined relationship index (CRI). If genetically linked loci are used then haplotype frequencies must be employed.

All formulas for likelihood ratios can be manually calculated, although this is often complex and inconvenient, particularly for less direct relationships such as siblings, half-siblings, and distant relatives such as grandchildren, cousins, and so on. Ideally, kinship software that is designed and validated will derive and calculate the required formula for any scenario. Some simple kinship formulas can be found in the following tables and according to AABB standard, profiles are denoted by phenotype, rather than genotype:

Missing Child Where Both Parents Have Provided a Sample

Mother	Possible Child	Father	Formula
A	A	AB	$1/2a^2$
A	AB	AB	1/4ab
A	AB	BC	1/4ab
AB	A	AB	$1/4a^2$
AB	A	AC	$1/4a^2$
BC	AB	AB	1/8ab
BC	AB	AC	1/8ab
BD	AB	AC	1/8ab
A	A	A	$1/a^2$
AB	A	A	$1/2a^2$
B	AB	A	1/2ab
BC	AB	A	1/4ab
AB	AB	AC	1/8ab
AB	AB	A	1/4ab
AB	AB	AB	1/4ab

Missing Father Where the Child and the Child's Mother Have Provided a Sample

Mother	Child	Possible Father	Formula
A	A	AB	1/2a
A	AB	AB	1/2b
A	AB	BC	1/2b
AB	A	AB	1/2a
AB	A	AC	1/2a
BC	AB	AB	1/2a
BC	AB	AC	1/2a
BD	AB	AC	1/2a
A	A	A	1/a
AB	A	A	1/a
B	AB	A	1/a
BC	AB	A	1/a
AB	AB	AC	1/2(a+b)
AB	AB	A	1/(a+b)
AB	AB	AB	1/(a+b)
	AB	AC	1/4a
	AB	AB	(a+b)/4ab
	AB	A	1/2a
	A	AC	1/2a
	A	A	1/a

Missing Sibling Where a Half or Full Sibling is Available

Sibling	Possible Full Sibling	Formula
AB	AB	(1 + a + b + 2ab)/8ab
A	A	$(1+a)^{2/}(2a)^2$
A	AB	(1+a)/4a
AB	AC	(1+2a)/8a
AB	CD	1/4

Half Sibling	Possible Half Sibling	Formula
AB	AB	(a + b + 4ab)/8ab
A	A	(1+a)/2a
A	AB	(1+2a)/4a
AB	AC	(1+4a)/8a
AB	CD	1/2

Mutation LRs

In mass fatality operations that use kinship analysis, mutations will be routinely encountered due to the large number of meioses and the large number of loci that are tested. A method for incorporating genetic inconsistencies into the statistical analysis should be decided upon and well understood before testing begins so that mutations are reported in a consistent manner. By choosing the methods in advance, the DNA operations will minimize potential accusations of biased interpretations. A complete description of currently available mutation calculation methods for paternity and maternity cases can be found in Appendix D. Regardless of the method chosen, the mutation calculation must be included in the CRI . See Appendix D for more information on incorporating mutations in complex kinship scenarios.

2. Combined Relationship Index

When comparing the DNA profiles of a set of human remains and a pedigree of possible relatives, the kinship index will usually be calculated at 15 or more loci. The CRI is the mathematical product of the likelihood ratios of each individual locus reported.

$$CRI = Index(FGA) \times Index(TPOX) \times Index(vWA) \times \ldots$$

C. Combining DNA and Non-DNA Information

1. Posterior Odds

Posterior odds (PO) is merely the kinship index multiplied by the prior odds. The posterior odds provide a numerical weight to the opinion of identification. The mathematics for the combination of the kinship index and the prior odds is as follows:

$$Posterior\ Odds = Likelihood\ Ratio \times Prior\ Odds = CRI \times P$$

2. Probability of Relationship

The probability of relationship (posterior probability) allows one to render an opinion about a relationship in understandable terms for the general public. The probability of the relationship expressed as a percentage is calculated by the following equation:

$$Probability\ of\ Relationship = PO/(PO+1) \times 100$$

or

$$Probability\ of\ Relationship = (CRI \times Pr\ /[CRI \times Pr + (1-Pr)]) \times 100$$

where

PO = Posterior Odds, Pr = Prior Probability and CRI = Combined Relationship Index

*In cases of related victims, often multiple LRs and prior probabilities will need to be evaluated to properly resolve the case. This requires substantial specialized expertise on the part of the individual performing the matching and the reporting.

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Appendix C. Inclusion Probability

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8

Positive Evidence of Paternity Calculated According to Essen-Möller: The Bayesian Approach

Jack Valentin, PhD

BACKGROUND

Blood typing in a paternity case can lead to either of two results: the tested man is excluded, or he is not excluded. If he is not excluded, he is possibly the father. How *probable* is it that he is the father?

There are several different approaches to this question. The theoretical exclusion capability, given the mother-child combination, has the advantage of being easy to grasp. Unfortunately, it is not a sufficient measure, ie, it does not extract all information available in data. In some cases, it is quite misleading.[1] A much better alternative is the likelihood ratio, first suggested in this context by Erik Essen-Möller.[2] This measure is sufficient, efficient (has minimum variance) and consistent (estimates improve with increasing sample size).

Other methods that have been suggested are usually claimed to be easier to calculate and/or grasp, at the expense of being insufficient. Such simplified measures can, eg, be likelihood ratios calculated without any regard to genetic complications such as dominance.[3] Another kind of suggestion is to "normalize" the likelihood ratio by multiplying with the exclusion capability.[4]

BAYES' THEOREM IN PATERNITY TESTS

Formally, there are two alternative hypotheses in a one-man case with one child: H_0 = the man is the biological father, and H_1 = he is not the father. There are now three different sorts of probability involved. First, the two hypotheses have prior probabilities of being correct, regardless of the phenotypes observed. Second, there are conditional probabilities of the male phenotype being what it is, the condition being either that H_0 is true or that H_1 is true. And finally, there is a posterior probability for each hypothesis that this is true.

Bayes' Theorem states that the posterior probability of H_0 being correct is the product of the prior probability of H_0 and the conditional probability of the male phenotype given H_0, divided by the sum of this product and the corresponding product for H_1. In symbolic notation, where R is the

observed male phenotype with due regard to mother and child:

$$P(H_0|R)=\frac{P(H_0)\cdot P(R|H_0)}{P(H_0)\cdot P(R|H_0)+P(H_1)\cdot P(R|H_1)} \quad \ldots (1)$$

The prior probability is a difficult point. In one sense, a realistic prior probability can be obtained from filed cases. A simple, if not very efficient, estimator of $P(H_0)$ in one-man cases is the number of cases without exclusion divided by the total number of cases times the theoretical exclusion rate. Thus, in Sweden $P(H_0)$ in one-man cases ≈ 0.75.

But this takes no account of individual circumstances. Obviously, the prior probability of the tested man's paternity is higher if the couple live together than if she is a prostitute and he a customer. Even if data on coition dates, birth measures, etc., are available, they are difficult to convert into probabilities, particularly since so much of this is based on statements rather than facts.

European paternity experts are usually convinced that the judge, not the blood typing expert, should be responsible for evaluation and use of a realistic prior probability.[5–7] Thus if the blood typing expert computes a "probability of paternity", ie $P(H_0|R)$, he resorts to Bayes' Postulate: when realistic prior probabilities cannot be established, neutrality is maintained by substituting equal prior probabilities for all hypotheses. Thus, (1) reduces to

$$P(H_0|R)=\frac{P(R|H_0)}{P(R|H_0)+P(R|H_1)} \quad \ldots (2)$$

LIKELIHOOD RATIOS

Division of both numerator and denominator of the above expression by the same quantity of course does not change the value obtained. Essen-Möller [2] realized that combination of data, say from several blood group systems, would be easier if numerator and denominator were divided by $P(R|H_0)$. Thus, with his notation where $P(R|H_0)=X$ and $P(R|H_1)=Y$,

$$P(H_0|R)=W=\frac{1}{1+Y/X} \quad \ldots (3)$$

The quantity Y/X is termed a likelihood ratio. Likelihood ratios for different blood group systems can be multiplied directly to form a combined likelihood ratio.

An improvement [8] to avoid cumbersome multiplication is to perform intermediate calculations with so-called EM values = log (Y/X)+10. For Whites, very extensive EM tables are published.[9–14]

As an alternative,[15] one can divide numerator and denominator by $P(R|H_1)$ instead of $P(R|H_0)$.

$$W=\frac{X/Y}{X/Y+1} \quad \ldots (4)$$

Of course, the posterior probability calculated according to (4) is identical to that obtained by (3). The likelihood ratio X/Y, often termed the Paternity Index, is the odds in favor of paternity, and has the subjectively pleasing property that it increases as the probability of paternity increases.

CALCULATION—A WORKED EXAMPLE

Assume that the protein polymorphism Gc is tested. There are two codominant alleles, 1 and 2, and consequently three distinct phenotypes: 1–1, 2–1 and 2–2 (so-called subtyping is not used in this example). The allele frequencies are p for allele 1 and q for 2, so with Hardy-Weinberg equilibrium the phenotype frequencies are p^2, 2pq and q^2. In Scandinavia, $p \approx 0.75$ and $q \approx 0.25$.

Let the mother be 1–1, the child 2–1 and the man 2–1. The man is not excluded from paternity, so we proceed to calculate a likelihood ratio. Several approaches are possible, but here a generalized method, easily transferred to a computer algorithm, will be used even if other methods would lead to less calculation work in this particular example with its simple genetics.

The relationship between the tested persons under the two hypotheses of paternity or nonpaternity is best illustrated with three pedigrees (Fig. 8-1). The first pedigree, M-C-Pf, assumes that the putative father is also the biological father. The second, M-C, pedigree shows mother and child assuming that the man is not the true father, and the third, Pf, shows the man also assuming that he is not the father. The probabilities of obtaining these three pedigrees are as follows:

$$P(\text{M-C-Pf}) = p^2 \cdot \tfrac{1}{2}(2pq) = p^3q \quad \ldots (5a)$$

$$P(\text{M-C}) = p^2 \cdot q \quad \ldots (5b)$$

$$P(\text{Pf}) = 2pq \quad \ldots (5c)$$

Essen-Möller's original, verbal definition of $P(R|H_0) = X$ was: the frequency of the tested man's phenotype among true fathers, and of Y: the frequency of his phenotype among random men. Using the pedigrees, he thus defined $X = P(\text{M-C-Pf})/P(\text{M-C})$ and $Y = P(\text{Pf})$. Okajima [16] suggested the alternative definitions $X = P(\text{M-C-Pf})$ and $Y = P(\text{M-C}) \cdot P(\text{Pf})$. Both sets of definitions lead to identical likelihood ratios, but Okajima's definition will be used since it has the advantage that X is the frequency with which the corresponding likelihood ratio occurs among fathers, Y the frequency with which it occurs among nonfathers.

Either of the two sets of definitions leads to the Paternity Index,

$$X/Y = p^3q/(p^2q \cdot 2pq) = 1/(2q) = 2 \quad \ldots (6)$$

Substituting into (4) leads to a posterior probability of paternity of $W = 2/(2+1) = 0.67$. For comparison, we can also compute the theoretical exclusion capability of this mother-child combination: the child must have received "2" from its father, so all 1–1 men are excluded. The exclusion capability is thus $p^2 = 0.56$.

LIKELIHOOD RATIO INTERPRETATION

To illustrate different decision methods, the entire set of cases in a population is represented by a unit square (Fig. 8-2, this and Figs. 8-3 through 8-5 are modified

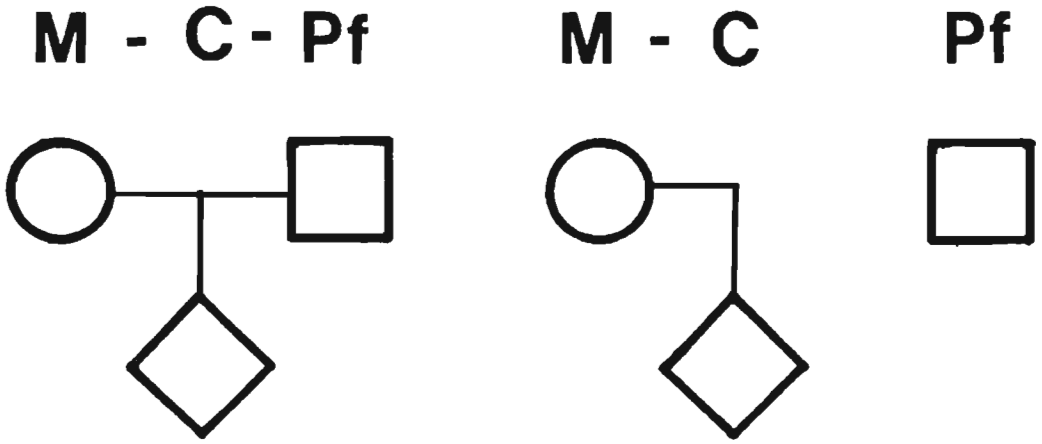

Fig. 8-1—The three pedigrees involved in a case of disputed paternity.

from Baur and Rittner [5]). Along the X axis, the square is divided into two subsets with true fathers, column width = $P(H_0)$, and nonfathers, column width = $P(H_1)$. Along the Y axis, each column is divided into segments corresponding to the various phenotype combinations that can occur. The height of each segment corresponds to the frequency of that phenotype combination among fathers or among nonfathers. In other words, the segment height is X in the column with true fathers and Y in the column with nonfathers. The order of phenotype combinations, and therefore of X and Y segments, is such that paternity exclusions, with X = 0 and Paternity Index = 0, are at the top, and each X/Y ratio is bigger than the one above it and smaller than the one below it in the figure.

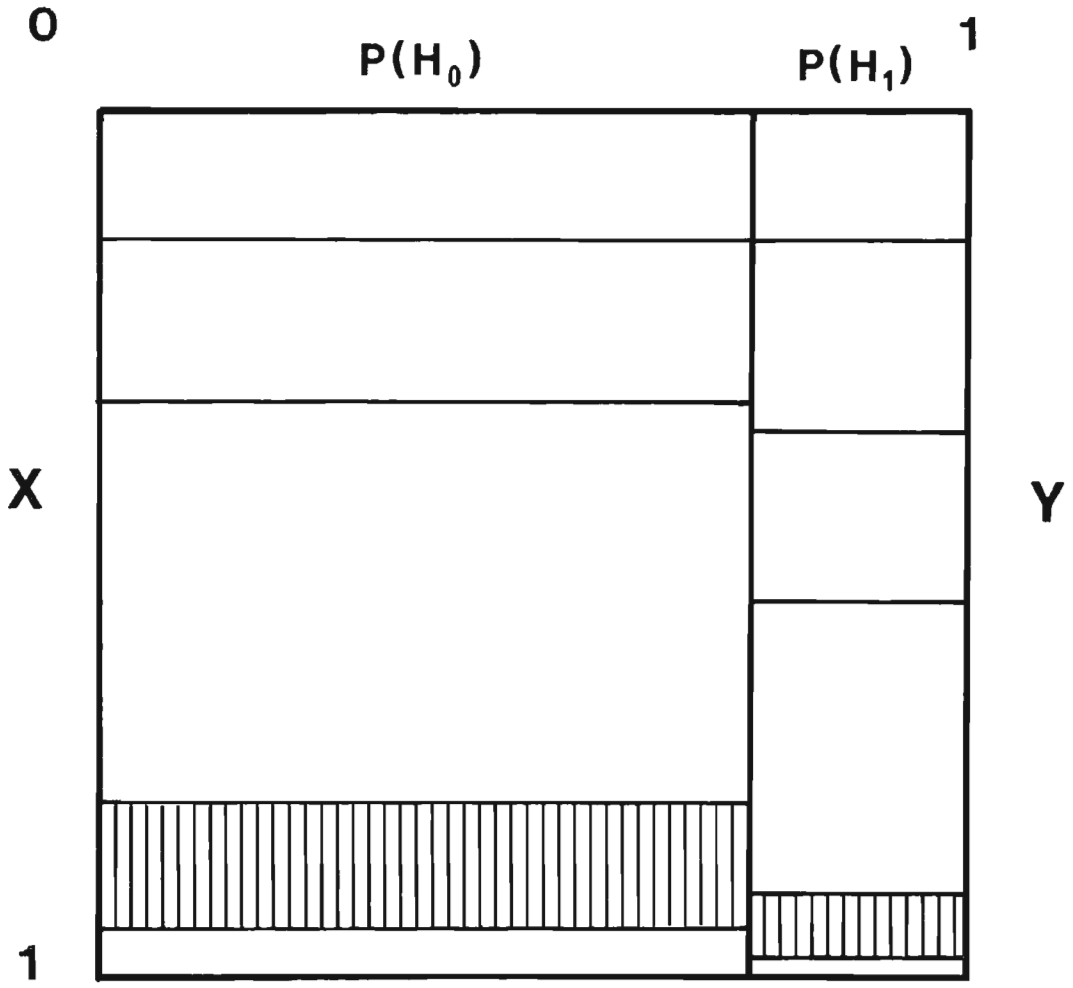

Fig. 8-2—A unit square representing all paternity cases. The square is divided into two columns representing cases involving the true father (prior probability = $P[H_0]$) and cases with nonfathers. Each segment in a column represents a particular group of phenotype combinations, with height $P(R|H_1)$ = X for fathers, Y for nonfathers. To scale for p = 0.75; the striped segments correspond to heterozygous men passing the rare allele to their children, ie, the Paternity Index X/Y = 2.

The Bayesian Approach

The posterior probability in the Bayesian sense in the worked example turned out to be 0.67. This was computed assuming Bayes' Postulate of equal prior probabilities. In fact the prior probability can be quite different from 0.5. The difference between the computed and a realistic posterior probability is the difference between comparing segment heights and comparing segment areas (Fig. 8-3). There is no way to avoid the effect of the prior probability. Nevertheless, many authors have tried to suggest decision procedures which are purportedly prior-independent.

The Neyman-Pearson Approach

An important method in decision-making is based on Neyman-Pearson's lemma, which shows that for a given significance level, there is always a critical region that minimizes the frequency of Type II errors. This is a very valuable method, but it does not eliminate the problem of prior probabilities, and it is not necessarily useful in paternity cases.

Applied to the worked example, Neyman-Pearson analysis involves a discussion of the errors that would occur if the present likelihood ratio were chosen as a decision limit for future cases. If in future cases with the same systems tested we find a Paternity Index of 2 or higher, the decision will be *paternity;* if the Paternity Index is less than 2 the decision will be *nonpaternity*. What is the total error frequency?

Type I, or α, error occurs if a true H_0 is rejected. In the unit square, this is repre-

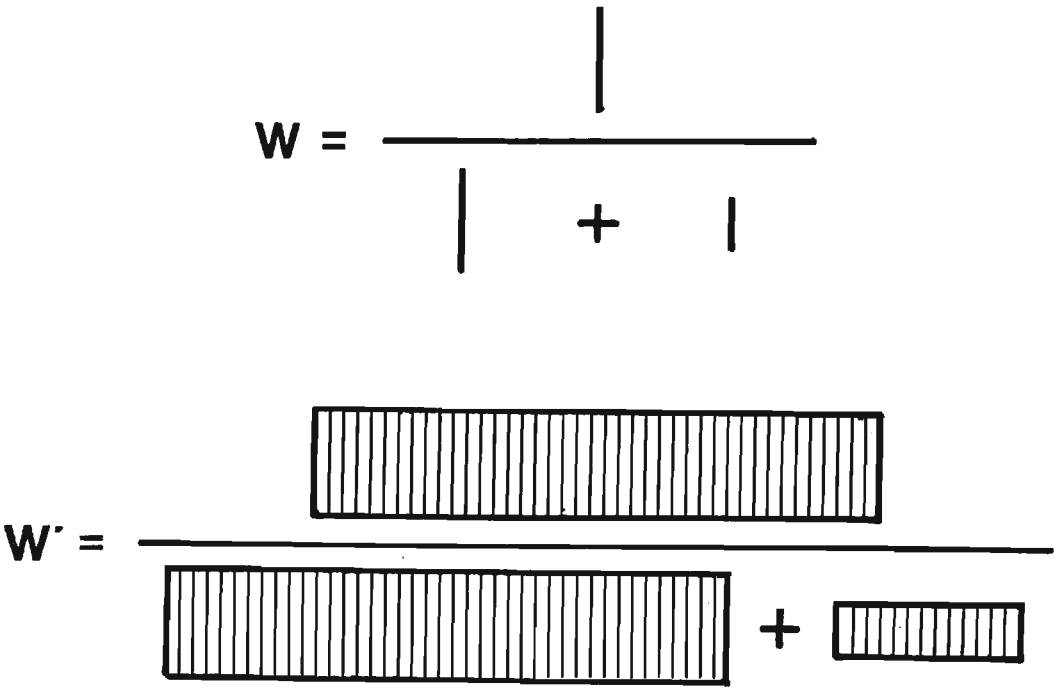

Fig. 8-3—Bayesian computation of the posterior probability of paternity. If prior probabilities are disregarded, the principle is to compute the relative frequency of the phenotype combination involved among such cases where the man is the true father, ie, the height of the corresponding X segment from Fig. 8-2 is divided by the sum of the heights X + Y. If a realistic prior is involved, then the areas, rather than heights, of the segments are used. This leads to a realistic posterior probability W' (= 0.86 if $P[H_0] = 0.75$ as assumed in the figures).

sented by all segments in the X column that are above the segment corresponding to the observed Paternity Index 2. Type II, β, error corresponds to accepting a false H_0 and is represented by the segments in the Y column below and including that corresponding to the Paternity Index 2. These areas are shaded in Fig. 8-4.

The sizes of α and β depend on the allele frequencies p and q, and as such these quantities can be calculated without knowledge of the prior probabilities. However, as the figure shows, they are *conditional* probabilities that refer only to the subsets of fathers and nonfathers, respectively. Unless the prior probabilities are known, it is *not* possible to estimate the *total* error rate.

Variations of a Theme

Kataja [4] suggests normalization of the Paternity Index by multiplication with the exclusion capability for the mother-child combination in question. Such a normalized Index would have a constant expectation for any given combination of tests, and would be a statistically more amenable variable than the likelihood ratio as it is. If analyses were always to be done by Neyman-Pearson methodology, normalization according to Kataja would probably be recommendable. Unfortunately, the normalized Index cannot be directly converted into a Bayesian posterior probability.

In one version of the Neyman-Pearson test,[17] the frequency of β, Type II, errors is

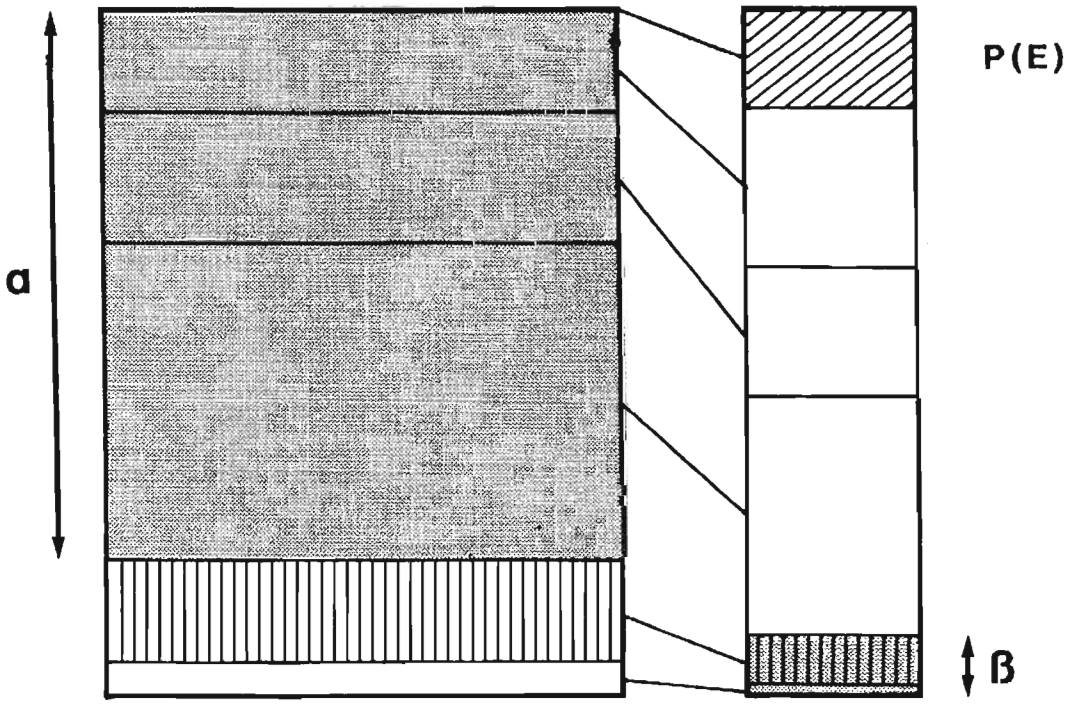

Fig. 8-4—Neyman-Pearson interpretation of the paternity case. If X/Y = 2, corresponding to the striped segment, is chosen as a decision limit, the shaded areas correspond to erroneous decisions. Some phenotype combinations, exclusions, occur only among nonfathers and with a frequency P(E) which is the average exclusion capability of the system.

calculated for *nonexcluded* nonfathers only. Thus, the height of the Y column is reduced from 1 to 1−P(excl) (Fig. 8-5). Since the exclusion capability depends on the number of systems tested, this means that error rates computed in this way cannot be compared directly. In selected cases the relative size of this error rate can be very big even if its absolute size is very small, so that the untrained user will get a completely erroneous impression of the situation when error rates are presented.

Neyman-Pearson Methods: Some Problems

We have seen here that Neyman-Pearson analysis does not solve the problem of prior probability. Of course, it might still be argued that the method has advantages over Bayesian tests. At this logical level, a number of authors have sided with Bayesianism, against Neyman-Pearson methods in paternity tests.[1, 5, 8] Irrespective of one's view at that level, there are, however, some obvious pragmatic problems. Thus, it will be very difficult to use Neyman-Pearson methods in complicated cases involving other than mother, child, and one man, all from the same population. When HLA is analysed, Neyman-Pearson methods appear impossibly difficult to use.

CALCULATION—MORE COMPLICATED CASES

More Than One Man

Bayes' Theorem can accommodate an infinite number of hypotheses. For sim-

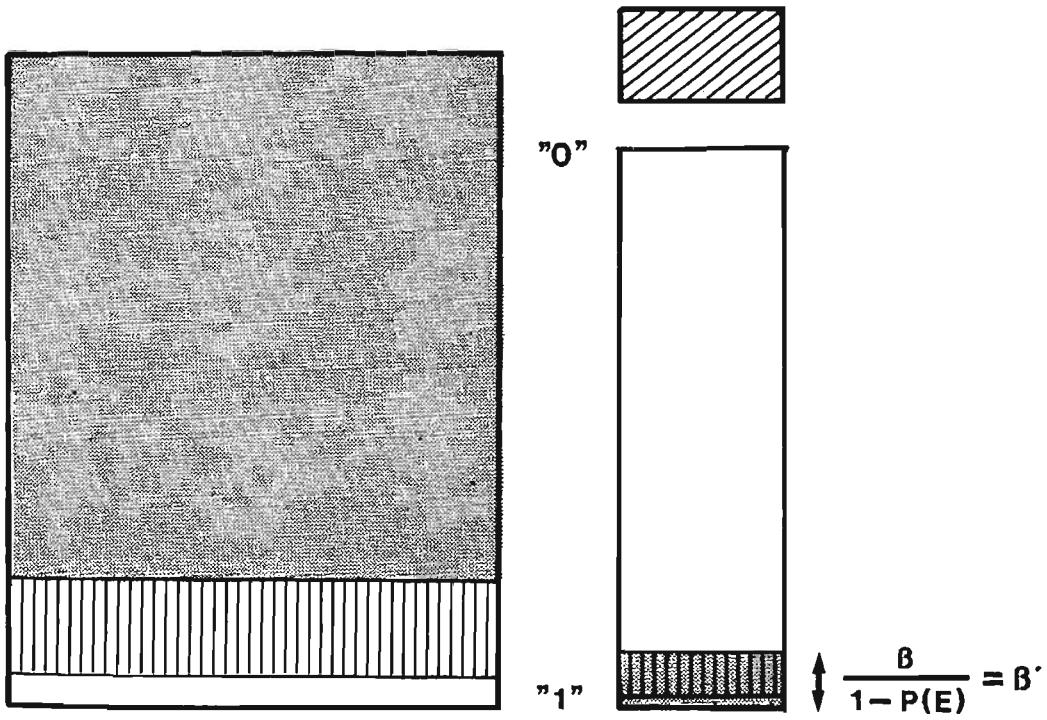

Fig. 8-5—Modified Neyman-Pearson analysis. The β, Type II, error frequency is computed only among nonexcluded nonfathers.

plicity, we adhere to Bayes' Postulate of equal prior probabilities. Adjustment for more than two hypotheses is achieved by summing all $P(R|H_i)$ in the denominator of expression (2). A corresponding adjustment is also very simple when one works with likelihood ratios as in (4), as shown in the following example.

Assume that we study the same three people as in the previous Gc example, but two further men are tested. Their phenotypes are 2–1 and 2–2. There are now four different hypotheses to consider: H_0 = the first man is the father, H_1 = the second man, H_2 = the third man, and H_3 = a fourth, unknown and/or untested man is the father. The probabilities of observing the three mens phenotypes under these hypotheses come out as $P(R|H_i)=X_i=P(\text{M-C-Pf}_i)\cdot\prod_{j=1} P(Pf_j)$:

$$\begin{aligned} X_0&=p^2\cdot\tfrac{1}{2}(2pq)\cdot 2pq\cdot q^2=2p^4q^4\\ X_1&=p^2\cdot 2pq\cdot\tfrac{1}{2}(2pq)\cdot q^2=2p^4q^4\\ X_2&=p^2\cdot 2pq\cdot 2pq\cdot q^2\quad=4p^4q^4 \end{aligned}\qquad \ldots(7)$$

Similarly, $P(R|H_3)=Y=P(\text{M-C})\cdot\prod_{i=0}^{3} P(Pf_i)$:

$$Y=p^2\cdot q\cdot 2pq\cdot 2pq\cdot q^2\quad=4p^4q^5 \qquad \ldots(8)$$

There are two alternative philosophies here. Either, one assumes that only the three tested men are possible as fathers. In that case, expression (2) gives the posterior probability of the i:th man as $X_i/\Sigma X$. Or, the possibility of a fourth "unknown" man is admitted. Then expression (2) becomes $X_i/(\Sigma X+Y)$. Thus, we need two alternative denominators:

$$\Sigma X=8p^4q^4 \qquad \ldots(9)$$

$$\Sigma X+Y=8p^4q^4+4p^4q^5 \qquad \ldots(10)$$

Substituting expressions (7) and (9) into (2), we find that the actual probability of paternity of the first man comes out as $2p^4q^4/8p^4q^4=0.25$, if only the three tested men are possible as fathers. The allele frequencies do not influence the calculations, since one of three persons with known genotypes is the father; the first man carries one of totally four Gc^2 alleles present in the three men so his probability of having supplied this is $\frac{1}{4}=0.25$.

Similarly, if we do admit that an unknown man could be the father, we substitute (7) and (10) into (2) and the probability of paternity of the first man becomes $2p^4q^4/(8p^4q^4+4p^4q^5)=2/(8+4q)=0.22$.

Just as in a one-man case, one can divide numerator and denominator of (2) by the same amount. Dividing by Y leads to the following likelihood ratios:

$$\begin{aligned} X_0/Y&=2p^4q^4/(4p^4q^5)=1/(2q)\\ X_1/Y&=2p^4q^4/(4p^4q^5)=1/(2q)\\ X_2/Y&=4p^4q^4/(4p^4q^5)=1/q \end{aligned}\qquad \ldots(11)$$

and $Y/Y=1$. Notice that these are *the same* likelihood ratios that apply in one man cases. Thus, tabulated likelihood ratios for one-man cases can be applied *directly* in cases with two or more than two men. As above, there are two alternative denominators depending on if "unknown" men are possible as fathers:

$$\Sigma(X/Y)=1/(2q)+1/(2q)+1/q=2/q \qquad \ldots(12)$$

$$\Sigma(X/Y)+Y/Y=2/q+1 \qquad \ldots(13)$$

In analogy with expression (4), the posterior probability of paternity for the first man using (11) and (12) or (13) becomes either $[1/(2q)]/(2/q)=0.25$, or $[1/(2q)]/(2/q+1)=1/(4+2q)=0.22$. Obviously Paternity Indices tabulated for one-man cases give correct posterior probabilities also in multi-man cases.

If there are two unknown men, Y must be added twice to the denominator in expression (2), or $Y/Y=1$ must be added twice in (4). For n unknown men, it is added n times. The effect of this is exactly the same thing as if one assumes only one unknown man, but a higher prior prob-

ability of paternity is assigned to him than to the tested men.

Thus, the posterior probability of paternity of the i:th man can be stated in a quite general form to be

$$W_i = \frac{X_i/Y}{\Sigma(X/Y)+n} \qquad \ldots (14)$$

where n is the number of "unknown" men. (This is a different result than that obtained by Nijenhuis in this conference.)

If EM values, ie, $\log(Y/X)+10$, are used, extension to more than one man is described by Schulte Mönting and Hummel.[18] Their formula implies a prior probability of 0.5 for the unknown man and 0.5 for the *sum* of the prior probabilities of the tested men. This could exaggerate the prior probability of the unknown man. On the other hand, it is of course easy to adjust their formula so that all men, tested and "unknown," get the same prior probability (or any other combination of prior probabilities desired).

More Than One Child

In a one-man case with two children, there are four different hypotheses to consider, viz H_0 = the tested man is the father of both children, H_1 = he is father of the first child, H_2 = father of the second child, and H_3 = he is not at all the father. Calculation [19, 20] of conditional probabilities corresponding to X and Y is straightforward, and there are extensive tables of such conditional probabilities for White people.[10–13] Likelihood ratios for one-man, one-child cases can not be used. The big problem is perhaps the choice of prior probabilities. Bayes' Postulate of equal prior probabilities [19, 20] could be quite unrealistic here.

Mixed Races

We have tacitly assumed that all tested persons belong to the same population, this being the local population of the country where blood tests are made. If all tested persons are from another population, adequate allele frequencies for that population would normally be used. The most complicated case is that mother and man come from different populations. In construction of likelihood ratios from expressions (5) adequate frequencies should be used, but this is not a very simple instruction to follow for (5b), the probability of the mother-child phenotype. If the accused man is not the father, which is then the donor population for the child's paternal allele? In other words, does the true father belong to the tested man's, or to the mother's, or to some third, population?

It is particularly important to use adequate frequencies when mother and tested man come from different races.[21] Fortunately, the question of the paternal population is usually simple in such cases. For instance, if the mother is White and the child a Mulatto, the true father must be Black and thus the adequate frequency of the paternal allele is that for "Blacks." Of course, it might be very difficult or impossible to know the actual magnitude of this frequency.

Untested Persons And Information From Relatives

Sometimes, the mother, the accused man or both of them are unavailable for testing. Instead, relatives such as presumed grandparents may be at hand. (Of course such relatives are indispensable if the accused man cannot be tested.) In principle, the "phenotype" of the untested person(s) is constructed as a suitably weighted average of those genotypes that are possible, taking information from relatives into account.

Using these constructed "phenotypes," one can calculate suitable Paternity Indices or other likelihood ratios. Manual calcula-

tion is straightforward albeit sometimes cumbersome [22–25] and for Whites, there are tables of EM values for cases without the mother and cases without the accused man but with his two parents.[10–13] If many relatives are available, eg, several siblings to an untested person, all available information should be used in a Bayesian procedure when the new "phenotype" is constructed. For worked examples, see Hummel [26, 27] but note that in these references, Y/X ratios are erroneously used to obtain weighted average likelihood ratios. Only X/Y ratios can be treated this way (ie, weighted algebraic, but not harmonic, mean likelihood ratios can be used).

Incest, Close Relationship Between Mother And Putative Father

If the mother and the accused man are closely related to each other, as if the man is the mother's brother or father, they are not randomly sampled from the population. In such cases, one can also use the method of appropriate weighting to obtain the genotype composition of the phenotypes.[25] A similar reasoning can be applied if two tested men are closely related to each other. In practice, it would rarely be important to pay attention to any other than first degree relatives (ie, parents, children, siblings).

Linked Loci

For a single locus, one generation of random mating suffices to give Hardy-Weinberg equilibrium. When two, or more, loci are involved, disequilibrium may persist for long times. This means that the frequency of a gamete genotype can be different from the frequency obtained if the relevant allele frequencies are multiplied together. Close linkage is one of the mechanisms that enhance and prolong such disequilibrium. When linkage disequilibrium is known to occur, it is absolutely necessary to take haplotype ($\approx$ chromosome) frequencies into account.[28]

It is also necessary to consider recombination.[29] If disequilibrium is such that a haplotype is rarer than expected on the basis of the frequencies of the alleles that constitute this haplotype, failure to observe the effect of recombination can lead to enormous underestimation of the true probability of paternity, say 5% instead of 95%.[29] Of course, recombination occurs in the mother, in the accused man and in the population at large. Throughout all, calculations must be performed remembering all coupling/repulsion combinations that are possible for a phenotype and gamete production with and without recombination.

If adequate recombination frequencies are not available, then it simply is not possible to calculate the probability of paternity.

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DISCUSSION

DR. DENISE SALMON: This review of Bayes' Theorem application in paternity tests is a very clear one.

Some remarks can be added:—

1. *"More than one man" cases*

Jack Valentin presents the relation between posterior probability of paternity and likelihood ratios involving several men. Whichever hypothesis is involved, the first man versus two other men, or versus two other men and an unknown random man, we may notice that the posterior probability of paternity gives information about the whole hypothesis.

On the other hand, likelihood ratios are more often applied in these "several men" cases, by comparing two of these men, with the ratio of $\frac{X0}{X1}$, for example.

Actually, it seems that in 1982 "more than one man" cases must be simplified to one-man cases, extensive testing being available in up-to-date laboratories.

Complicated calculations on several men are not now needed.

2. *The Prior Probability*

The 0.5 prior probability is a better choice.

2.1. It is usual in Bayesian process to start from the equal uncertainty about hypotheses.

2.2 It is acknowledged in Bayesian decision making that, when more examinations on states of nature are performed, the least important determining the final result is the initial choice of probability. It is necessary to practice extensive testing, and the unrealistic choice of 0.50 gradually fades.

2.3 The Bayes' Theorem application with a 0.5 prior probability is a convenient transformation, allowing us to obtain a posterior probability. It is better, for comparison of results, that the different laboratories decide to start from the same prior probability, the neutralist one.

3. *Likelihood ratio or posterior probability.*

There is some issue about the choice. It must be admitted that the likelihood ratio is a better statistic than the posterior probability, for example, for definition of Type I and Type II errors.

If we consider the question on prior choice as resolved by preferring equal odds, the two modes of expression are nearly

equivalent, in my opinion. The only difficulty is that the expert must be consistent in his formulations; either he always expresses his results in a likelihood ratio, or more precisely in an index of paternity $\left(\frac{X}{Y}\right.$ being easier to understand than $\left.\frac{Y}{X}\right)$ or he always formulates answers in posterior probability form.

The Courts should be accustomed to one form of expression and we are sure that they will reach a sound decision from the blood results available.

DR. J. VALENTIN: In our experience, even using HLA and quite a lot of different systems, we do get not one but rather many cases where more than one man is left over and we simply cannot eliminate several men cases from our practical statistics. So I think we do need to have a formula and that it is important to use the correct formula for treating cases with several men.

DR. SALMON: Do you use iso-electric focusing of enzymes?

DR. VALENTIN: Yes.

DR. SALMON: It's a very efficient method.

DR. VALENTIN: We use iso-electric focusing for PGM and Gc but no matter how powerful the method you use you will always have the odd case where you do have two men or three men or 55 men. You'll always have something which doesn't fit into your model.

Appendix D. Mutations

During reference sample validation and kinship comparisons, there may be instances where the laboratory may encounter a genetic inconsistency at a particular locus within a family. It is important to recognize the possibility of mutation when a difference occurs at only one or two loci and is accompanied by a large residual index. The residual index is a combined index of all matching loci for a case. When the residual index is large, it is likely that the inconsistency represents either a true mutation or a relative of the missing person. The possibility that the inconsistency may be a result of inaccuracies in the reference sample relationships or possible DNA profiling error should be explored. It is most likely that a genetic mutation is the cause if no biological relationship or technical reason can be identified for the inconsistency. Mutations are known to occur when there is a change in DNA from one generation to the next at a particular locus. Studies have shown that mutation rates are locus- and gender-specific. A list of mutation rates can be found in Appendix 11 of the *AABB Guidance for Standards for Relationship Testing Laboratories*, 9th Edition. Mutation rates can also be found at http://www.cstl.nist.gov/strbase/mutation.htm. The current model suggest the most frequent (>90%) type of genetic inconsistency appears as an allele one repeat larger or smaller than the reference sample allele. In mass fatality operations that use kinship analysis, mutations will be routinely encountered due to the large number of meioses and the large number of locations that are tested.

When performing complex kinship analysis, it is not always possible to determine the source of the mutational event. An example would be when two siblings present themselves to identify a third and there is one locus where it appears the three siblings cannot have the same biological parents, when in fact they do. It is impossible to determine which child actually received the mutated allele. In these types of indeterminate mutational event cases, the methods outlined in the excerpt below may be insufficient. Currently there are no well-referenced publications that address these situations. One method of incorporating an indeterminate mutational event is to multiply the number of times that the mutational event could have occurred by the mutation index. Because the mutation could have been transmitted between the parents and missing person or the parents and one of the other siblings, there are two opportunities for the mutational event to happen.

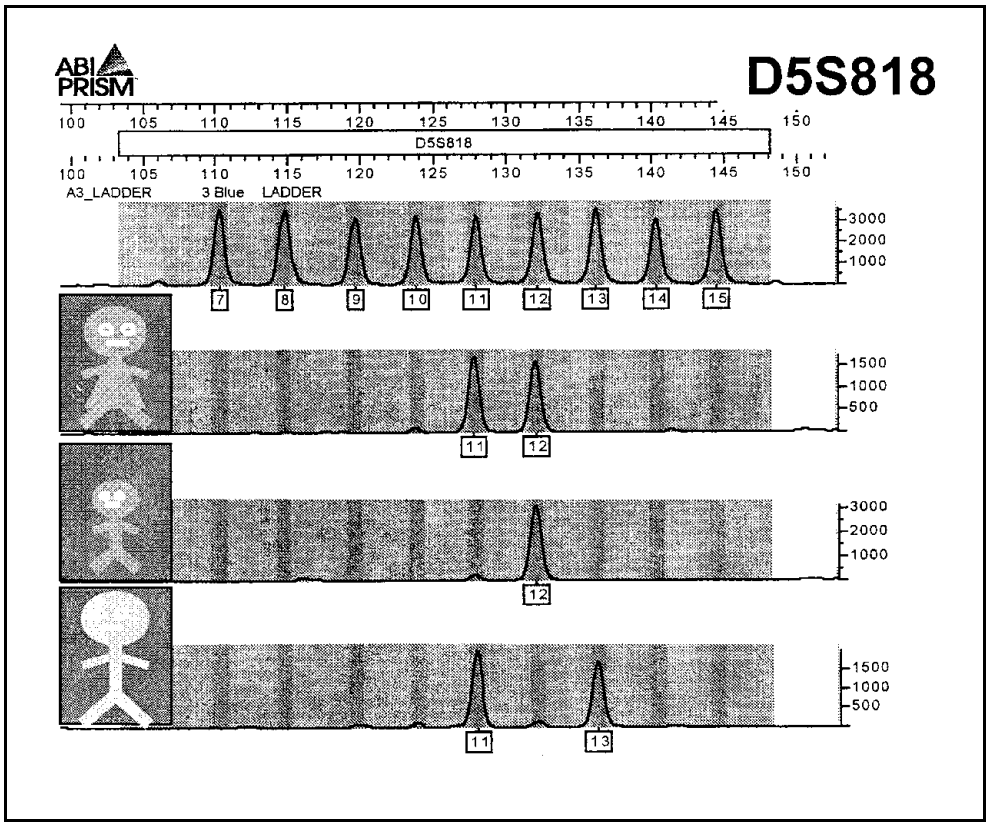
There are situations when the mutational event has no impact on the identification. In these instances, the individual with the mutation may be removed from the statistical calculation. An example would be where samples from a mother and three siblings were provided to identify remains and one of the siblings does not match the mother at one locus but shares a paternal allele with the other children. It is acceptable to exclude the inconsistent child at this locus or entirely.

The following is an excerpt from Appendix 12 of the AABB Guidance for Standards for Relationship Testing Laboratories, AABB 9th Edition (2009) on *The effect of isolated inconsistencies in the statistical evaluation of paternity: A 2005 update* by David W. Gjertson, PhD, pertaining to the incorporation of inconsistencies in relationship calculations.

Appendix 12. The Effect of Isolated Inconsistencies in the Statistical Evaluation of Paternity: A 2005 Update*

David W. Gjertson, PhD

When using DNA systems to resolve paternity disputes, a rare but problematic outcome can arise due to isolated single-locus mismatches among many tested systems between the alleged father and child. Since mutations can produce "rogue" DNA fragment lengths capable of being transmitted to an offspring, an opinion of non-paternity is unjustified in the standard case, especially when all remaining systems yield a large combined paternity index (residual PI). For typical DNA-RFLP and -STR systems, average mutation rates (μ) range from 0.005% to 1% (see Appendix 11). As pointed out by Fimmers et al[14] modifying the paternity likelihood for a possible mutation pattern presents a practical, rather than theoretical, obstacle: the problem of estimating parameters to describe specific mutational events.



The figure above illustrates a case whose subjects presented with an isolated mismatched system (D5S818) but otherwise had results consistent with paternity (residual PI = 1,774 in 7 systems).[3] In the D5S818 system, the child possesses the 12-repeat unit allele (figure's middle profile), which does not

*This appendix is adapted, with permission, from a previous version based on two presentations—one by D. Endean and D. Gjertson to the Statistics Workshop in Scottsdale, Arizona, September 1996, sponsored by the Promega Corporation and another by D. Gjertson to the English Speaking Working Group of the International Society for Forensic Genetics in Orlando, Florida, October 1996.
†Data kindly supplied by Dr. John Taddie of Long Beach Genetics.

match either of the tested man's alleles (figure's bottom profile), hence a single-locus mismatch. Rarely, however, one of the tested man's fragments (11 or 13) may mutate to produce an allele with 12 repeat units. (For completeness, the true father may transmit an unknown/null allele to the child, but the tested man's fragments may mutate to produce this allele also.)

Suppose $m_{I,J}$ equals the specific mutation rate for changing allele I to J where I and J come from the set of DNA fragments. (Note that $m_{I,I}$ represents the rate of non-mutation transition.) Following instructions from Fimmers et al's[16] instructions and ignoring multiple mutational events and silent (unknown/null) alleles, the specific mutation PI for the illustrated case is roughly equal to

$$PI \approx \frac{m_{13,12} + m_{11,12}}{2 f_{12}}$$

where f_{12} represents the frequency for the D5S818-12 allele in a defined population. As stated above, current estimates for $m_{I,J}$ are equivocal for DNA systems—a situation that has forced laboratories to use varied approaches to incorporate mutations into biostatistical evaluations.

RFLP Systems

Especially problematic are RFLP mutation events. They lack precise estimation because of the 1) degree of polymorphism in most RFLP systems, and 2) presence of fragment-band measurement error (ie, small mutations in DNA fragment size may be indistinguishable from resolution limitations causing some mutations to be missed and not counted). Therefore, the use of the specific mutation rate formula for PI has been avoided in RFLP systems.

Alternatively, an average mutation PI ($\overline{PI}$) can be derived using μ and substituted to compute an overall result. Initially, one tallies the frequency of cases displaying a single paternal discrepancy in the isolated system relative to a battery of DNA loci. The $\overline{PI}$ numerator equals this frequency, $\hat{\mu}$, since either the banding pattern is consistent with paternity or it isn't. (Practically speaking, $\hat{\mu}$ counts those cases where the paternal bands in the single isolated system lie beyond the child's bands by more than the system's delta value.) Under nonpaternity, the $\overline{PI}$ denominator depends on the chance of a correct exclusion. Hence, this probability equals the average probability of exclusion for nonfathers ($\overline{PE}$). Average PEs can be empirically estimated or calculated by general equation; see Garber and Morris[11]; Brenner and Morris[4]; and Endean[5]. Then, the average mutation PI is given by

$$\overline{PI} = \frac{\hat{\mu}}{\overline{PE}}.$$

The following table shows some average mutation PIs for RFLP systems tested with Hae III at Long Beach Genetics:

RFLP System	Average Paternal Mutation Rate, $\hat{\mu}$	$\overline{PE}$	$\overline{PI}$
YNH24/D2S44	0.0005	0.90	0.0006
TBQ7/D10S28	0.0009	0.92	0.0010
EFD52/D17S26	0.0018	0.91	0.0020
PH30/D4S139	0.0059	0.90	0.0066

In practice, average mutation PIs should be calculated by each laboratory using its empirically validated data since values can vary depending on experimental conditions. Although the average mutation PI can be combined with remaining systems to obtain an overall result, one needs to be aware that, when using an average substitute, one is not making use of all of the information and the actual mutation PI may be different for different banding patterns. To minimize the effects of possible extraneous PI values, one may use low estimates for the average mutation rate and routinely require that additional systems be tested for paternity after encountering isolated mismatches. The natural consequence is that, if the tested man is truly not the biological father of the child, the probability of finding additional non-matches leading to an opinion of exclusion is increased.

PCR/STR Systems

The assessment of mutation events should be easier with PCR/STR technology as the repeat differences between obligatory alleles and alleles in disputed parents can be tallied during the evaluation process. Empirical distributions suggest that most STR mutational events involve few repeat alterations (>90% of reported mutations involved a ± 1 repeat-unit difference; see Appendix 3 of the 2003 Annual Report and Brinkman et al[15] for details). Also, Dawid et al[16] have examined several mutational models incorporating biological processes to express the probability of each allele-specific transition in terms of μ and a set of adjustable parameters. This group of investigators favored models that assumed the probability of a transition from one allele to another decreases as the difference between their sizes increases.

Although some laboratories use methods that incorporate the number of repeat differences when computing PI, no widespread consensus exists regarding the exact formulation—even the same laboratory may employ different strategies for different types of cases. According to the 2003 Annual Report, four methods for STR mutations are in typical use: 1) the average mutation rate ($\hat{\mu}$) as the PI (8% of laboratories); 2) the average mutation rate divided by the average probability of exclusion (61%, ie, the RFLP solution); 3) the average mutation rate as a transmission frequency (6%); and 4) Brenner's method in looking at the repeat length difference between STR alleles (18%). The remaining 7% of laboratories reported multiple or no methods.

Brenner[3] suggested the following hypothetical distribution for the number of repeat units altered during STR mutation events:

- 50% of all mutations increase by one step
- 50% decrease by one step
- 5% increase by two steps
- 5% decrease by two steps
- 0.5% increase by three steps
- 0.5% decrease by three steps
- ... etc.

(Note it is inconsequential that these values sum to more than 100% since they will be subsequently multiplied by small mutation probabilities.) A general change of s-steps equals $(1/10)^{s-1}$.

Brenner's method can be illustrated using the figure's case. In the D5S818 system, the mother (MO) has alleles 11,12, the child (CH) was 12, and the tested man (TM) was 11,13. The tested man's alleles 11, 13 were 1 step smaller and 1 step larger than the child's allele 12, respectively. The *PI* numerator X is given by

X = Pr (phenotypes|true trio) = Pr(CH|MO,TM, true trio) × Pr(TM,MO|true trio)
= Pr (CH|MO,TM, true trio) × Pr(TM,MO).

where CH, MO and TM represent the child's, mother's and tested man's phenotypes, respectively. X's last term is independent of the child's paternity, and X is proportional to

Pr(CH|MO, TM, true trio =)
Pr(11,12 mother transmits 12) × Pr(11,12 man transmits 11)
× Pr(mutation) × Pr(1 step change|mutation) × Pr(mutation increases length|1-step mutation)
+ Pr(11,13 man transmits 13)
× Pr(mutation) × Pr(1 step change|mutation)×Pr(mutation decreases length|1-step mutation)

$$= \left(\frac{1}{2}\right)\left(\left(\frac{1}{2}\right)\mu\left(\frac{1}{10}\right)^{1-1}\left(\frac{1}{2}\right)+\left(\frac{1}{2}\right)\mu\left(\frac{1}{10}\right)^{1-1}\left(\frac{1}{2}\right)\right)= \left(\frac{1}{4}\right)\mu.$$

where μ is the average mutation rate for the D5S818 locus. Next, the PI denominator Y equals the probability of the observed phenotypes given a false trio:

Y = Pr(phenotypes|false trio) = Pr(CH|MO, TM, false trio) × Pr(MO,TM)

Assuming no mutational events are needed to "explain" the observed alleles given a false trio, Y is proportional to

Pr(CH|MO, TM, false trio) = Pr(11, 12 mother transmits 12) × Pr(untested man transmits 12)
= $(\frac{1}{2})(f_{12})$

where f_{12} represents the frequency for the D5S818-12 allele in a defined population. Thus,

$$PI = \frac{X}{Y} = \frac{\mu}{2f_{12}}$$

Based on the 2003 Annual Report, $\hat{\mu} = 0.00116$ and $f_{12} = 0.384$ for the D5S818 system. Thus PI = 0.00116/0.768 = 0.002. In many situations, a tested man's phenotype is composed of a "proximal" allele and a "distal" allele relative to a single child's obligatory allele. Under these circumstances, one can ignore the distal allele, and $X \approx \frac{1}{4}\mu\left(\frac{1}{10}\right)^{s_p-1}$ where s_p is the step change for the proximal allele. Now,

$$PI_{1-step} = \frac{\mu}{4f_{obligatory}}$$

and

$$PI_{2-step} = \frac{\mu}{40f_{obligatory}}$$

etc ...

where $f_{obligatory}$ represents the frequency for the child's obligatory allele.

Further Considerations

In 2002, the AABB began collecting data on specific STR mutation changes. Laboratories were asked to list apparent mutation changes from one allele to another. For counts, laboratories were instructed to assume that the mutation involved the closest allele. Undoubtedly biased, these counts can nonetheless be used to illustrate procedures for deriving specific allele transition rates.

Table 1 shows observed counts, empirical and smoothed probabilities of paternal D5S818 allele transitions (ie, estimates of $m_{i,j}$). Empirical values were deduced from published allele frequencies (Butler et al[17]) and meioses/mutations reported in the 2003 annual report. Smoothed values were a type of Bayes estimate based on a lognormal distribution for the relative deltas (quotient of cell frequencies divided by the product of marginal frequencies obtained from observed counts). Smoothed estimates were altered toward the product of the marginal frequencies. The alteration had little effect on established transitions but increased rates where no direct mutations had been tallied, especially among transitions from relatively low frequency alleles to high frequency ones. Although "smoothing" introduced some robustness of estimation, the data were still potentially biased as mentioned, and some distal transition probabilities seemed overly inflated. Conceivably, one should be able to use raw phenotypic constellations (including consistent and inconsistent cases) of all non-excluded trios to generate maximum likelihood estimates of specific allele transitions via the EM algorithm (ie, similar to iterative gene-counting method pioneered by Ceppellini et al.[18] Additionally, the smoothing concept may be improved by introducing plausible biological processes (models) for *a priori* distributions into an empirical Bayes solution.

Using the smoothed $m_{i,j}$ values in the table, the PI for our illustrated case equals

$$PI = \frac{m_{13,12} + m_{11,12}}{2 f_{12}} = \frac{.00026 + .00130}{2(0.384)} = 0.002.$$

Both methods (Brenner[13] and Fimmers et al[14]) yield similar values for PI. For comparison, $\overline{PI} = 0.0025$ since $\hat{\mu} = 0.00116$ and $\overline{PE} = 0.4618$ for the D5S818 system. Generally, however, one expects these methods to yield different *PI* values, especially when the proximal transition involves alleles that are 2+ repeat units away from one another.

So far, we have addressed the issue of incorporating paternal mutational events into the biostatistical evaluation of paternity. However, cases exist where postulating a maternal mutation helps to resolve a paternal inconsistency. For example, consider the following trio of D5S818 phenotypes: a mother with repeat-unit alleles 9,13; a child with 12,13; and a tested man with 11,13. The child possesses the 12-repeat unit allele, which does not match either of the tested man's or mother's alleles. Previously, we considered the situation akin to the mother transmitting the 13-repeat unit allele and one of the tested man's fragments (11 or 13) mutating to produce an allele with 12-repeat units. However, we should also consider the possibilities that one of the mother's fragments (9 or 13) mutated to produce the 12-repeat unit allele and the tested man transmitted the 13-repeat unit allele.

Both Brenner's and Fimmers's methods are easily adapted to incorporate maternal mutational events into *PI* formulas. Straightforward extensions of their instructions yield the following formulas for our example:

$$PI_{Brenner} = \frac{\frac{1}{4}[\mu_P + \mu_M(\frac{1}{200} + \frac{1}{2})]}{\frac{1}{2} f_{12}} \approx \frac{\mu_P + (\mu_M/2)}{2 f_{12}}$$

where μ_M and μ_P are the average maternal and paternal mutation rates for the D5S818 locus; and

$$PI_{Fimmers} = \frac{m^*_{9,12} + m^*_{13,12} + m_{13,12} + m_{11,12}}{2 f_{12}}$$

where $m^*_{I,J}$ and $m_{I,J}$ equal the maternal and paternal specific mutation rates for changing allele I to J. Brinkmann et al observed that, for STR loci, male mutational events are 4-5 fold more frequent than female events. Based on 2003 annual report data for the D5S818 system, $\hat{\mu}_m = 0.00025$ and, $\hat{\mu}_p = 0.00116$ so $PI_{Brenner} = 0.002$. (In this case, the corrected and "paternal-only" *PIs* would be practically equal—a sensible answer given the relatively low maternal mutation rate for D5S818 and the number of steps separating mother's/tested man's alleles from those of the child. This is not always the case, and maternal mutations warrant consideration by laboratories when evaluating inconsistencies.) Finally, one could estimate gender-specific $m_{I,J}$ using the procedures listed above and substitute corresponding values into a corrected $PI_{Fimmers}$.

Table 1: Paternal D5S818 Allele Transitions

D5S818 counts, $c_{I,J}$

		Child										
		7	8	9	10	11	12	13	14	15	16	Total
Father	7	255										255
	8		509									509
	9	1		7637	3							7641
	10			1	7892	3						7896
	11			1	5	55506	14					55526
	12				1	11	59040	40				59092
	13						28	21582	40			21650
	14							26	987	6		1019
	15								3	251	1	255
	16									2	253	255
	Total	256	509	7639	7901	55520	59082	21648	1030	259	254	154098

Empirical mutation probabilities, $m_{I,J}$

		Child										
		7	8	9	10	11	12	13	14	15	16	Total
	7	1										1
	8		1									1
	9	0.00013		0.99948	0.00039							1
	10			0.00013	0.99949	0.00038						1
Father	11			0.00002	0.00009	0.99964	0.00025					1
	12				0.00002	0.00002	0.99912	0.00068				1
	13						0.00129	0.99686	0.00185			1
	14							0.02552	0.96860	0.00589		1
	15								0.01176	0.98431	0.00392	1
	16									0.00784	0.99216	1

Smoothed mutation probabilities, $m_{I,J}$

		Child										
		7	8	9	10	11	12	13	14	15	16	Total
	7	0.98619	0.00300	0.00120	0.00120	0.00240	0.00240	0.00180	0.00060	0.00060	0.00060	1
	8	0.00151	0.99125	0.00181	0.00090	0.00121	0.00151	0.00090	0.00030	0.00030	0.00030	1
	9	0.00014	0.00012	0.99865	0.00040	0.00020	0.00020	0.00014	0.00006	0.00004	0.00004	1
	10	0.00004	0.00006	0.00014	0.99891	0.00039	0.00020	0.00014	0.00006	0.00004	0.00004	1
Father	11	0.00001	0.00001	0.00002	0.00009	0.99954	0.00026	0.00004	0.00001	0.00001	0.00001	1
	12	0.00001	0.00001	0.00003	0.00002	0.00019	0.99902	0.00068	0.00002	0.00001	0.00001	1
	13	0.00002	0.00002	0.00005	0.00005	0.00009	0.00130	0.99657	0.00185	0.00002	0.00002	1
	14	0.00015	0.00015	0.00045	0.00045	0.00076	0.00091	0.02555	0.96553	0.00590	0.00015	1
	15	0.00060	0.00060	0.00121	0.00121	0.00241	0.00241	0.00181	0.01145	0.97468	0.00362	1
	16	0.00060	0.00060	0.00120	0.00120	0.00241	0.00241	0.00181	0.00060	0.00783	0.98133	1

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Additional Resources

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Appendix E. Discrepancies between Social and Genetic Pedigrees

Kinship analysis in mass fatality identification projects will routinely encounter discrepancies between reported pedigrees and the true genetic kinship, revealing, for example, instances of nonpaternity. This information could be damaging to the families. It is important to realize the project may not have the authority and/or consent to provide that information to the affected people, even though that information may affect the ability to make a DNA identification. The DNA operations should not release inconsistent social information to families without careful and thorough consideration of its effects. The donor consent statement needs to consider and address whatever mechanism will be used to handle pedigree inconsistencies. The policy on releasing this information should follow any local laws governing public access to information.

At a minimum, the entity making the final identification should be made aware of the nature of any discrepancies and the potential impact on the final identification, assuming that adequate confidentiality and data protection safeguards exist at that level. All logical explanations for the inconsistency need to be explored and explained thoroughly to the entity making the identification.

For example, if two children are provided to identify a missing father and a male victim has been identified as the father of one child and not the other, is it possible that this is an alternative father instead of the reported father. It is tempting to conclude the reported missing father has been identified, but there are two fathers in this case and it is not clear which one has been identified.

Appendix F. Family Pedigree and Sample Collection

Before samples are collected, it is important to generate a family pedigree for each reported missing (RM) case to identify which family references should be collected for testing. Most often, a representative from the DN unit will talk with family members and create the family pedigree or family tree. Because there may be confusion as to how family members are biologically related, it may be necessary to draw a family pedigree several different times. Family members may provide different or conflicting information on family biological relationships. Conflicting results may be resolved by speaking with other family members (See Appendix E). Once genetic profiles from the families have been generated and compared, additional information about the family structure can be obtained. In order to maintain a clear understanding of the family structure throughout the DNA operations, standard genetic nomenclature should be used to document the family structure. See Figure 1 for common pedigree symbols.

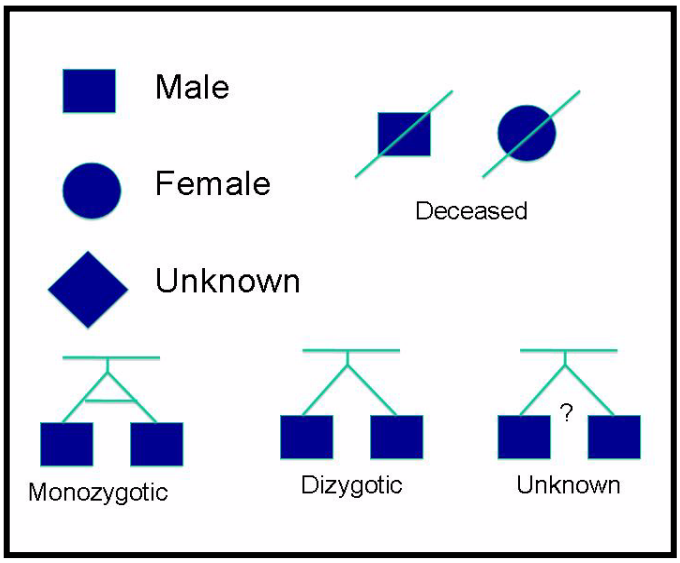

Figure 1: Common Pedigree Symbols

Figure 2 depicts how a family pedigree is drawn using standard pedigree symbols indicating mating individuals and offspring.

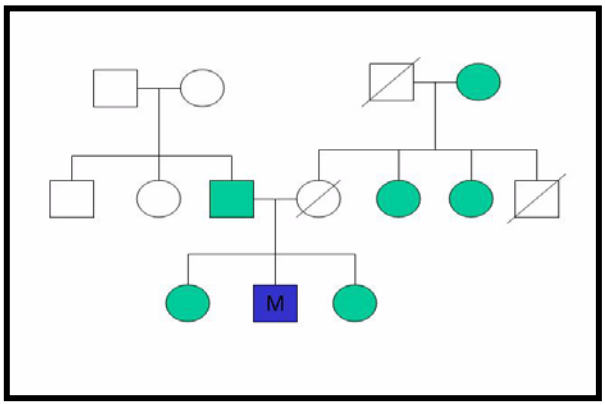

Figure 2: A Family Pedigree

Once a family pedigree of potential donors has been constructed, it is best to examine the pedigree carefully to determine which family reference samples to collect. The optimal or useful samples for each family will be dependent on the particular family structure and the availability of individuals providing the sample. The green highlighted individuals in Figure 2 indicate the individuals to be collected. To identify which samples to collect, it is necessary to follow the lines along the pedigree, starting from the RM, to each biological relative able to provide a sample. Samples from individuals beyond those providing a sample will not contribute additional genetic information and need not be collected. Figure 3 depicts an example family pedigree. The individuals in blue represent those who should be collected. If possible, all living offspring should be collected. In this example, a sample from the only living son would be collected. A sample from the son's other parent would also be collected to eliminate their genetic contribution to the offspring. Samples from the child's son and his mother would not be collected because they will not contribute any additional genetic information beyond what the son will contribute.

Next, the collector would follow the lines to the RM's biological parents, which should both be collected if available. In this case, only a sample from the father would be collected because the mother is deceased. Because the father's sample would be collected, samples from other family members passing through this paternal line, such as his siblings or parents, would not be collected.

If a parent is deceased or unavailable to provide a DNA sample, an attempt to gather his or her genetic information should be made by collecting samples from genetic relatives. In this case, samples would be collected from any of the mother's living siblings, parents, and children as shown.

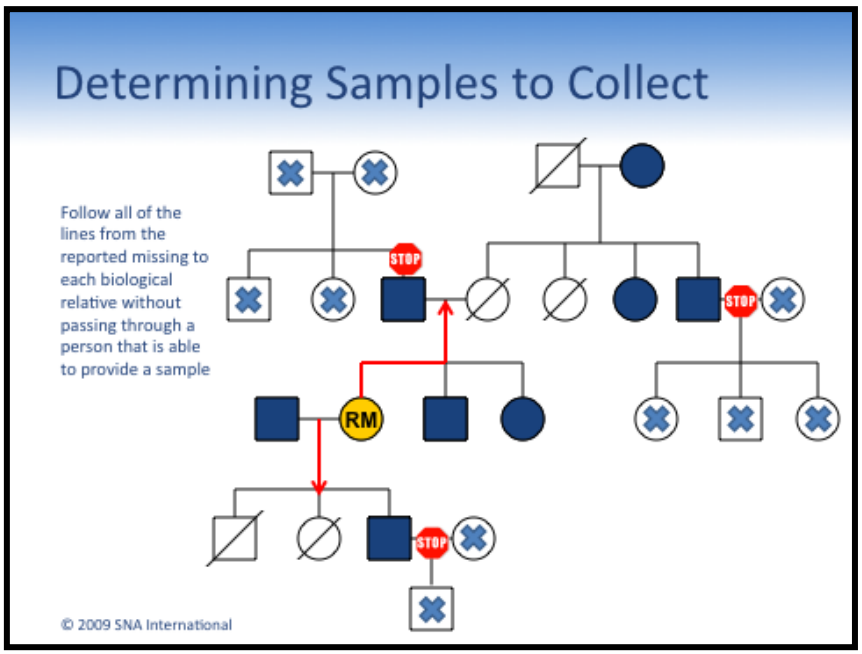

Figure 3. Determining Which Samples to Collect

Because family reference samples are collected from multiple individuals, it is important that all samples collected for the RM are placed in the same RM case.