TECHNICAL NOTE

Arvind K. Chaturvedi,[1] Ph.D.; Nicole T. Vu,[1] Ph.D.; Roxane M. Ritter,[1] M.S.; and Dennis V. Canfield,[1] Ph.D.

DNA Typing as a Strategy for Resolving Issues Relevant to Forensic Toxicology*

REFERENCE: Chaturvedi AK, Vu NT, Ritter RM, Canfield DV. DNA typing as a strategy for resolving issues relevant to forensic toxicology. J Forensic Sci 1999;44(1):189–192.

ABSTRACT: To investigate aircraft accidents, multiple postmortem biological samples of victims are submitted to the Civil Aeromedical Institute for toxicological evaluation. However, depending upon the nature of a particular accident, their body components are often scattered, disintegrated, commingled, contaminated, and/or putrefied. These factors impose difficulties with victim identification, tissue matching, and consequently authentic sample analysis and result interpretation. Nevertheless, these limitations can be overpowered by DNA typing. In this regard, three situations are hereby exemplified where DNA analysis was instrumental in resolving a tissue mismatching/commingling issue, pinpointing an accessioning/analytical error, and interpreting an unusual analytical result. Biological samples from these cases were examined for six independently inherited genetic loci using polymerase chain reaction (PCR) suitable for analyzing degraded DNA generally encountered in putrefied/contaminated samples. In the first situation, three of five specimen bags from one accident were labeled with two different names. DNA analysis revealed that one of these bags actually had commingled specimens, originating from two different individuals. Therefore, the sample was excluded from the final toxicological evaluation. In the second situation, an unacceptable blind control result was reported in a cyanide batch analysis. By comparing DNA profiles of the batch samples with those of the known positive and negative blind controls, it was concluded that the error had occurred during the analysis instead of accessioning. Accordingly, preventive measures were taken at the analytical level. The third situation was related to the presence of atropine at toxic concentrations in the blood (318 ng/mL) and lung (727 ng/g) with its absence in the liver, spleen, and brain. DNA analysis of the blood and liver samples exhibited their common identity, ensuring that the submitted samples had indeed originated from one individual. The selective presence of atropine was attributed to its possible administration into the thoracic cavity by the emergency medical personnel at the accident site for resuscitation, but circulatory failure prevented its further distribution. These examples clearly demonstrate the applicability of the PCR-based DNA typing to enhance the effectiveness of forensic toxicology operation.

KEYWORDS: forensic science, forensic toxicology, DNA typing, polymerase chain reaction, tissue mismatching, cyanide analysis, atropine administration, aircraft accident investigation

Acquiring accurate and authentic analytical data on biological evidence to seek the chemical basis for the cause of accident or death is the main objective of forensic toxicology laboratories. For achieving that objective, strict adherence to quality assurance/quality control (QA/QC) procedures is essential. Such adherence allows not only the correct scientific interpretation, but also the judicial admissibility, of analytical results with a high degree of confidence. Nevertheless, the accuracy of the results resides with the integrity of the submitted biological samples. This perspective is of particular importance in aircraft accident investigations, wherein multiple types of postmortem specimens are collected for analysis from the victims, and depending upon the nature of an aircraft accident, victim bodies are frequently scattered, disintegrated, commingled, contaminated, and/or putrefied. The complications associated with the identification of remains and matching of tissues frequently hamper investigation, including toxicological evaluation. However, such complications are effectively resolved in our laboratory by DNA typing. Since environmental and biological factors responsible for putrefaction also damage DNA (1), the genetic material is potentially degraded in postmortem samples generally obtained from aviation accident sites.

Analysis of degraded DNA by the conventional restriction fragment length polymorphism (RFLP) technique is not as effective as it is by the method based on the polymerase chain reaction (PCR), a directed *in vitro* DNA synthesis. The former is more suitable for fresh samples containing nondegraded, high molecular weight DNA. This technique is also relatively cumbersome and time-consuming (2). The PCR-based analysis has been successfully employed for analyzing minute amounts of degraded, low molecular weight DNA in contaminated and putrefied samples (2–4). Thus, the PCR analysis was adopted in the present study.

Besides the identification of victims and of postmortem tissues, we apply DNA typing in resolving other postmortem toxicology issues, such as accessioning/analytical errors and interpretation of unusual analytical results. Covering the whole spectrum of forensic

[1] Research Chemists, Toxicology and Accident Research Laboratory, Aeromedical Research Division, Civil Aeromedical Institute, Federal Aviation Administration, U.S. Department of Transportation, Oklahoma City, OK.

* This document is disseminated under the sponsorship of the U.S. Department of Transportation in the interest of information exchange. The United States Government assumes no liability of the contents or use thereof. This paper has also been approved for production as an internal technical report of the Office of Aviation Medicine, Federal Aviation Administration, U.S. Department of Transportation.

Received 16 March 1998; and in revised form 29 May 1998; accepted 1 June 1998.

Copyright © 1999 by ASTM International

toxicology operation, three situations—tissue mismatching/commingling, accessioning/analytical error, and unusual analytical finding—are hereby exemplified to illustrate the effective applicability of the DNA analysis in addressing those issues. In these situations, identification of the samples was determined by target-DNA amplification and DNA hybridization using sequence-specific probes. During this process, loci associated with six independently inherited genetic markers were examined (5). These markers were human leukocyte antigen (HLA) DQA1, low-density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8 (locus on chromosome 7 linked with cystic fibrosis), and group specific component (GC). The combined discriminating power of these genetic markers statistically established the fidelity of the submitted biosamples in the three described situations.

Situations

Tissue Mismatching/Commingling

In a biohazard shipping box, five bags containing postmortem tissues from an aircraft accident, involving two occupants, were submitted for analysis. The types of tissues were liver, lung, kidney, and muscle; all samples appeared to be putrefied. Out of these five bags, two bags were clearly marked: The name of one victim was written on one bag, while the name of the other victim on the second bag. On the other hand, doubting the exact origin of the tissues, the remaining three bags were labeled with the names of both victims. Samples from the five bags were analyzed for DNA to establish the source of the tissues so that toxicological analyses could effectively be performed on the appropriately identified samples.

Accessioning/Analytical Error

A batch comprising a total of seven blood samples was accessioned and submitted for cyanide analysis. It entailed five aircraft accident-related case samples and blind controls (one negative and one positive). Review of the batch analysis revealed that the positive blind control was reported as negative. Thus, the batch was rejected. Since it was not certain whether the error occurred during the accessioning or the analysis, attempts were made to identify the source by subjecting all seven samples of the existing cyanide batch for DNA analysis. Along with these were analyzed the known positive and negative controls from which the blind controls for the batch were initially prepared; it was presumed that both controls originated from different biological sources. The observed DNA profiles in the batch samples were compared with those of the known controls.

Unusual Analytical Finding

Multiple specimens from an aircraft accident victim were submitted for analytical toxicology. Routine analysis disclosed the presence of atropine at unusual toxic concentrations in the blood and lung samples, but the drug was not detected in the liver, spleen, and brain samples. The presence of atropine in the biological samples could be related to three possible scenarios: intentional or accidental atropine poisoning, atropine administration by emergency healthcare providers into the thoracic cavity to modify the vasovagal activity, and tissues originating from different subjects. To verify the tissue origin, DNA analysis was performed on two selected tissue types (blood and liver), because atropine was present in the blood but absent in the liver—a primary drug metabolism site.

Materials and Methods

Materials

All reagents were of analytical grade, and the solvents were of chromatographic grade. These reagents and solvents, immunoassay and DNA analysis kits, standards, internal standards, derivatizing agents, and other necessary supplies were obtained from commercial sources. Different immunoassay kits for drug screens were purchased from Abbott Laboratories (Abbott Park, IL), Roche Diagnostic Systems (Nutley, NJ), and Diagnostic Products Corporation (Los Angeles, CA). The DNA analysis kits (Quantiblot™ Human DNA Quantitation Kit; AmpliType® PM + DQA1 PCR Amplification and Typing Kit) were supplied by Perkin-Elmer Corporation (Foster City, CA). Bovine serum albumin (BSA) of 96 to 99% purity was purchased from Sigma Chemical Company (St. Louis, MO).

Routine Forensic Toxicology

Submitted samples were analyzed for drugs, volatiles, carboxyhemoglobin (COHb), and cyanide using standard laboratory procedures. For drugs, various immunoassays and chromatographic techniques were used. Samples were analyzed for volatiles by headspace-gas chromatography. COHb was measured spectrophotometrically. Blood cyanide was determined colorimetrically using the chloramine-T/pyridine/barbituric acid reagent.

DNA Extraction

DNA from whole blood and other tissue samples was extracted in the presence of Chelex® 100 resin (Bio-Rad Laboratories, Richmond, CA) following the procedure of Walsh et al. (6). To 3 to 30 mg of each of the samples, 200 μL of the 5% Chelex® resin suspension in water was added, and the mixtures were incubated in a water bath at 56°C for 30 min and then at 90 to 100°C for 8 min.

DNA Quantitation

A chemiluminescent procedure was used to quantitate the amount of DNA in the obtained extracts by using Quantiblot™ Human DNA Quantitation Kit. Each 5 μL-extract was processed following the recommendations of the manufacturer (7). Chemiluminescent signals were detected on Hyperfilm™-ECL (Amersham Life Science Inc., Arlington Heights, IL) after 30 min exposure and development using a QX-70 medical film processor (Konica Medical Corporation, Wayne, NJ). The intensity of the sample signals was visually compared with that of the DNA standards processed simultaneously. This method selectively determines human DNA semiquantitatively in the range of 0.15 to 10 ng. Based on the DNA amounts, appropriate volumes of the extracts were utilized in the subsequent DNA amplification.

DNA Amplification

In vitro DNA replication by *Taq*-polymerase was carried out according to the manufacturer's directions for DQA1 and polymarker (PM) amplification (5). The reaction mixture also contained

160 μg/mL BSA to minimize inhibition of amplification by inhibitors present in the extracts. Target regions of six genetic loci were synthesized from 2 to 10 ng of the extracted DNA in a thermal cycler (GeneAmp PCR System 9600, Perkin Elmer Corporation, Foster City, CA) in 32 cycles. Of the six loci, one locus was DQA1. The remaining loci were PM: LDLR, GYPA, HBGG, D7S8, and GC. All amplified samples were kept at −70°C until typed.

PCR Product Verification, Hybridization, and Detection

Following the procedure for verification of PCR amplification (5), gel electrophoresis was performed on PCR products using an agarose preparation (Agarose for the Separation of GeneAmp™ PCR Products, Perkin Elmer Corporation, Foster City, CA) and a Horizontal Mini-Gel Electrophoresis unit (Sigma-Aldrich Techware, St. Louis, MO). The amplified products were evaluated by using a 4% agarose gel and visualized with ethidium bromide staining using a UV source (U. V. P., Inc., San Gabriel, CA). Hybridization and detection of the amplified DNA were also carried out as per manufacturer's recommendation (5).

Results and Discussion

In our present approach, six independently inherited genetic loci are included. They are considered to be sufficient for establishing the fidelity of the samples in question within the scope of a particular situation. According to Perkin-Elmer's population genetic studies, the power of discrimination for these loci ranges from 99.93 to 99.98%, depending upon the ethnic background of an individual (5). Although such discriminating power may not be as compelling as that generally cited for a matching within an ethnic population, it will be statistically sufficient for matching within a particular forensic toxicology situation involving a relatively well-defined, limited number of samples.

Tissue Mismatching/Commingling—From Table 1, it is clearly evident that the samples in Bag 1 and Bag 2 originated from different individuals, as both samples had different genotypes. The genotype of the specimen in Bag 2 was consistent with that of the specimen in Bag 5, confirming that these samples originated from the same individual. Results from the sample in Bag 3 were inconclusive, as out of six loci, only four loci could be successfully visualized, DQA1 and LDLR could not be typed. The ''C'' dot on DQA1 strip and ''S'' dot on PM strip were also not developed. The reamplification with an increased amount of DNA or the organic extraction (8) in place of Chelex® did not produce any conclusive typing results. The preferential amplification of the alleles in the sample from Bag 3 was further supported by the visibility of only the corresponding four DNA bands in the PCR product gel electrophoresis. Such partial amplification could be attributed to the presence of some unknown contaminant(s)/inhibitor(s) in the Bag 3 sample, and could further be ascribed to the quality (degraded) of DNA present in the sample (putrefied). It has been established that, in samples wherein DNA is degraded, some but not all alleles are amplified (9). Loci associated with longer basepair sequences are more affected than those with shorter basepair sequences. The number of basepairs at the six loci decrease in the following order: DQA1 (239/242) > LDLR (214) > GYPA (190) > HBGG (172) > D7S8 (151) > GC (138). The visibility of loci smaller than 214 basepair—GYPA, HBGG, D7S8, and GC—in the Bag 3 sample suggested probable degradation of the DNA fragments with longer sequences. Despite the limited typing, one can infer that the sample in Bag 3 possibly belonged to the same source as the sample in Bag 1, since the four visible loci in the former matched with that of the latter. The Bag 4 sample was a mixture containing biological material from two genetically different sources, since three alleles (1.2, 3, 4.1) on the DQA1 strip and imbalanced dot intensities on the PM strip were well evident. In a particular locus on the PM strip, an allele common in both Bag 1 and Bag 2 samples was more intense than the one present in only one sample (Table 1). Based on these DNA results, toxicological findings were reported on only those specimens determined to be from a single individual. Although toxicological evaluation failed to disclose the presence of commonly used drugs in either of the analyzed muscle samples (Bag 1 and Bag 5), 16 mg/dL ethanol was detected in the fluid from only the Bag 1 sample. Volatiles found in another tissue (Bag 4) were excluded from the reporting, as the sample was genetically determined to be a mixture of tissue originating from two different individuals.

Accessioning/Analytical Error—The DQA1 and PM genetic profiles of the samples in the cyanide batch, together with the known positive and negative blind controls, are presented in Table 2. DNA profiles of Sample 1 and Sample 2 matched with those of the known positive and negative controls, respectively. This finding is consistent with the accessioning records that Sample 1 was, indeed, the positive blind control and Sample 2, the negative blind control. These observations implied that the error did not take place during the accessioning or batch preparation but occurred during the analysis. Accordingly, proper corrective measures were taken at the analytical level to prevent the recurrence of such faulty analysis.

Unusual Analytical Finding—The concentrations of atropine found in various tissues of the victim are presented in Table 3. Atropine was present in the amounts of 318 ng/mL of blood and

TABLE 1—*DQA1 and PM profiles of samples from Situation 1.*

Bag	Specimen	Genetic Markers					
		DQA1	LDLR	GYPA	HBGG	D7S8	GC
1	Muscle	3, 3	AB	AA	AB	BB	AC
2	Liver	1.2, 4.1	AB	AB	AA	AB	CC
3*	Lung	—	—	AA	AB	BB	AC
4†	Kidney	1.2, 3, 4.1	AB	**A**B	**A**B	A**B**	A**C**
5	Muscle	1.2, 4.1	AB	AB	AA	AB	CC

* Dashes denote that the type at the locus was not detectable.

† Bold lettering in the sample profile indicates relatively high intensity dot observed within a particular locus.

TABLE 2—*DQA1 and PM profiles of the samples from Situation 2 and of the known positive and negative blind controls.*

Sample	Genetic Markers					
	DQA1	LDLR	GYPA	HBGG	D7S8	GC
1	1.2, 4.1	AB	AB	AB	AA	AC
2	1.1, 4.1	AB	AA	AB	AB	CC
3	4.1, 4.1	AB	BB	AA	AB	CC
4	2, 3	AB	AB	BB	AA	BC
5	2, 4.1	AB	BB	AA	BB	AB
6	1.2, 3	BB	AB	BB	AB	CC
7	1.2, 2	AB	AA	AB	AB	CC
Positive	1.2, 4.1	AB	AB	AB	AA	AC
Negative	1.1, 4.1	AB	AA	AB	AB	CC

TABLE 3—*Atropine concentrations in various tissues.*

Specimen	Atropine Concentration
Blood	318 ng/mL
Lung	727 ng/g
Liver	none detected
Spleen	none detected
Brain	none detected

TABLE 4—*DQA1 and PM typing results of the blood and liver fluid from Situation 3.*

	Genetic Markers					
Sample	DQA1	LDLR	GYPA	HBGG	D7S8	GC
Blood	1.2, 1.2	AB	AB	BB	AA	AA
Liver fluid	1.2, 1.2	AB	AB	BB	AA	AA

727 ng/g of lung. It was not detected in the other tissues (liver, spleen, and brain). The observed blood concentration was considerably higher than the lethal level (200 ng/mL) reported in an atropine poisoning case (10). The selective presence clearly demonstrated that the present case did not follow the general poisoning trend, wherein the agent should have been distributed throughout the body. The possibility of samples originating from different sources was ruled out by the DNA analytical results, as both blood and liver specimens were genetically identical (Table 4). Atropine's localized presence could be attributed to its administration by emergency medical care personnel directly into the thoracic cavity for resuscitation and to the circulatory failure preventing the drug's further distribution. This aspect is further supported by the case history that emergency medical personnel were at the aircraft crash site. Similar selective postmortem distribution patterns of lidocaine have also been reported in three cases following its endotracheal intubation for cardiopulmonary resuscitation (11).

In summary, the findings from the three exemplified situations clearly demonstrate the practical application and effectiveness of the PCR-based DNA typing in resolving some postmortem toxicology-related issues. This simple, less time-consuming typing procedure is suitable for the analysis of degraded DNA in the putrefied samples generally encountered in postmortem forensic cases, particularly those associated with aviation accidents. The molecular biology approach to address the forensic issues improves not only the degree of certainty but also the authenticity of toxicological results.

References

1. Budowle B, Baechtel FS, Adams DE. Validation with regard to environmental insults of the RFLP procedure for forensic purposes. In: Farly MA, Harrington JJ, editors. Forensic DNA technology. Chelsea, MI: Lewis Publishers Inc., 1991;83–91.
2. Sajantila A, Ström M, Budowle B, Karhunen PJ, Peltonen L. The polymerase chain reaction and post-mortem forensic identity testing: application of amplified D1S80 and HLA-DQα loci to the identification of fire victims. Forensic Sci Int 1991;51:23–34.
3. Hochmeister MN, Budowle B, Borer UV, Eggmann U, Comey CT, Dirnhofer R. Typing of deoxyribonucleic acid (DNA) extracted from compact bone from human remains. J Forensic Sci 1991;36: 1649–61.
4. Reynolds R, Sensabaugh G. Analysis of genetic markers in forensic DNA samples using the polymerase chain reaction. Anal Chem 1991;63:2–15.
5. AmpliType®, PM + DQA1 PCR amplification and typing kits, package insert, Roche Molecular Systems, Inc., Alameda, CA, 1995.
6. Walsh PS, Metzger DA, Higuchi R. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 1991;10:506–13.
7. Quantiblot™, Human DNA quantitation kit, package insert, Roche Molecular Systems, Inc., Alameda, CA, 1993.
8. Budowle B, Waye JS, Shutler GG, Baechtel FS. Hae III—A suitable restriction endonuclease for restriction fragment length polymorphism analysis of biological evidence samples. J Forensic Sci 1990;35:530–6.
9. Walsh PS, Erlich, HA, Higuchi, R. Preferential PCR amplification of alleles: mechanisms and solutions. PCR Methods Appl 1992;1: 241–50.
10. Baselt RC, Cravey RH. Disposition of toxic drugs and chemicals in man. 4th ed. Foster City, CA: Chemical Toxicology Institute, 1995;63–5.
11. Moriya F, Hashimoto Y. Postmortem diffusion of tracheal lidocaine into heart blood following intubation for cardiopulmonary resuscitation. J Forensic Sci 1997;42:296–9.

Additional information and reprint requests:
Dr. Arvind K. Chaturvedi
Toxicology and Accident Research Laboratory (AAM-610)
FAA Civil Aeromedical Institute
P.O. Box 25082
Oklahoma City, OK 73125-5066