Correspondence

Analytical Methods for Ignitable Liquid Residues

Sir:

While the comments of the Proficiency Advisory Committee that accompany each CTS Proficiency Test Report come with the caveat that "these comments are not intended to reflect the general state of the art within the profession," a little data analysis can certainly help the profession learn what works well and what doesn't work so well.

Such is the case with the "Flammables Analysis" tests distributed over the last three years. In comparing the performance of laboratories that use gas chromatography (GC) as their only instrumental technique, versus those who use gas chromatography/mass spectrometry (GC/MS), it is clear that those using GC/MS have a significantly higher probability of reporting results consistent with the manufacturer's specifications.

All three of the most recent tests have been straightforward identifications of neat liquids, or neat liquids plus water. In 1997, participants were asked to identify kerosene and diesel fuel (ASTM Class 4 and Class 5). In 1998, participants were asked to identify an isoparaffinic solvent (Class 0.2) and a naphthenic/paraffinic lamp oil (Class 0.5). In 1999, participants were asked to identify Kerosene (Class 4), a naphtenic/paraffinic lamp oil (Class 0.5), and mineral spirits (Class 3). Comparing the number of responses that were inconsistent with the manufacturer's identification with the method used yields the following data.

Year	Inconsistent Identifications Using GC Alone	Inconsistent Identifications Using GC/MS
1997 (1)	23/77 (30%)	20/132 (15%)
1998 (2)	19/42 (45%)	38/153 (25%)
1999 (3)	11/29 (38%)	39/173 (23%)

These results, while a cause for concern, do not address the ultimate forensic question asked of the participants. In 1998, participants were asked whether Item 1 could have had a common origin with Item 2, and all but one participant stated that the two were different from each other. In 1999, all but seven participants were able to distinguish the two liquids from each other, and only three of those (all GC users) reached the incorrect conclusion that the samples could have had a common origin.

One reason for the significant number of inconsistent answers may be confusion with the ASTM classification system. Some participants correctly named the products, then listed the wrong ASTM class number. In response to this problem, ASTM is discontinuing the use of class numbers, opting for the more descriptive class name.

Regardless of the reasons for the inconsistent answers, the above data show that laboratories that use GC/MS for identification of ignitable liquid residues have a significantly lower probability of misidentifying a substance compared with laboratories that rely on GC alone. In the light of this data, it seems to this scientist that it is time to consider the use of GC/MS for all identifications of ignitable liquids. The good news is that there has been a significant drop in the number of laboratories relying on GC alone, from 77 in 1997 to 42 in 1998, to only 29 in 1999. This trend should be encouraged.

John J. Lentini
Applied Technical Services, Inc.
1190 Atlanta Industrial Drive
Marietta GA 30066

References

1. Collaborative Testing Services, Flammables Analysis Report No. 9716, CTS, Herndon, VA, 1998.
2. Collaborative Testing Services, Flammables Analysis Report No. 9816, CTS, Herndon, VA, 1999.
3. Collaborative Testing Services, Flammables Analysis Test No. 99-536, CTS, Herndon, VA, 2000.

Commentary on: Sperling FAH, Anderson GS, Hickey DA. A DNA-based approach to the identification of insect species used for postmortem interval estimation. J Forensic Sci 1994;39:418–427 and on Vincent S, Vian JM, Carlotti MP. Partial sequencing of the cytochrome oxydase b subunit gene I: a tool for the identification of European species of blow flies for postmortem interval estimation. J Forensic Sci 2000;45:820–823.

Sir:

We wish to correct mistakes in recent and not so recent papers on DNA-based identification of forensically important insects. First, the sequence data reported for *Protophormia terraenovae* by Vincent et al. (1) (GenBank accession AF017426), as well as additional observations of *P. terraenovae* and the closely related *Phormia regina* (2), strongly suggested to us that the *P. regina* haplotype in Sperling et al. (3) (accession L14946) actually represents a misidentified specimen of *P. terraenovae.* A comparison of GenBank sequences as well as data from specimens of both species collected in California is shown in Fig. 1.

Re-examination of the original specimen supports this conclusion. Although the entire thorax was destroyed during the original study, the wings are intact and have the dark upper calypters characteristic of *P. terraenovae* and not *P. regina.* Investigators are ad-

```
                        22222222
                        66666677
                        24578823
                        59247879
P. reg. L14946          CTTCACTC
P. ter. Calif.          ....G..T
P. ter. AF017426        ....G..T
P. reg. Calif.          ACCT.TGA
```

FIG. 1—*Sequence Navigator (PE Biosystems) layout showing mtDNA sequence data from the gene for cytochrome c oxidase subunit I. Specimens were identified using morphological criteria as either* Phormia regina *(REG) or* Protophormia terraenovae *(TER). Base position numbers correspond to those used for* Drosophila yakuba *(4).*

vised to rely on GenBank accession AF262956 for identification of *P. regina.*

Second, Vincent et al. did not, as their title indicates, obtain sequence from "cytochrome oxydase b subunit gene I" but rather from the gene for cytochrome c oxidase subunit I (4,5), commonly referred to as simply "cytochrome oxidase I (COI)." We fear that readers will mistakenly conclude that Vincent et al. studied cytochrome b, which is so often used for the identification of vertebrate tissues.

References

1. Vincent S, Vian JM, Carlotti MP. Partial sequencing of the cytochrome oxydase by subunit gene I: a tool for the identification of European species of blow flies for postmortem interval estimation. J Forensic Sci 2000;45:820–3.
2. Wagner DH, Wells JD. An investigation of reported regional genetic variation in the forensically important blow fly, *Phormia regina*, using mitochondrial DNA. Proc Am Acad Forensic Sci 2000;6:181.
3. Sperling FAH, Anderson GS, Hickey DA. A DNA-based approach to the identification of insect species used for postmortem interval estimation. J Forensic Sci 1994;39:418–27.
4. Clary DO, Wolstenholme DR. The mitochondrial DNA molecule of *Drosophila yakuba:* nucleotide sequence, gene organization, and genetic code. J Mol Evol 1985;22:252–71.
5. Scheffler I. Mitochondria. New York: Wiley-Liss 1999.

J.D. Wells, Ph.D.
Department of Justice Sciences
University of Alabama at Birmingham
Birmingham, Alabama 35294, USA
Email: jwells@uab.edu

F.A.H. Sperling, Ph.D.
Department of Biological Sciences
University of Alberta
Edmonton, Alberta, T6G 2E9, Canada
Email: Felix.Sperling@ualberta.ca

Editor's Note: Dr. Stephane Vincent is in agreement with the comments made in the sentence beginning "Second, Vincent et al. did not, ..."

Future citations of the referenced published papers should read: Sperling FAH, Anderson GS, Hickey DA. A DNA-based approach to the identification of insect species used for postmortem interval estimation [published erratum appears in letter from Wells and Sperling, J Forensic Sci 2000(Nov);45(6)] J Forensic Sci 1994;39:418–27.

Vincent S, Vian JM, Carlotti MP. Partial sequencing of the cytochrome oxydase b subunit gene I: a tool for the identification of European species of blow flies for postmortem interval estimation [published erratum appears in letter from Wells and Sperling, J Forensic Sci 2000 Nov;45(6)] J Forensic Sci 2000;45:820–3.

Further commentary on: Wu AHB, Hill DW, Crouch D, Hodnett CN, McCurdy HH. Minimal Standards for the Performance and Interpretation of Toxicology Tests in Legal Proceedings. J Forensic Sci 1999;44(3):516–522 and on the commentary of Kidwell DA, Smith FP. J Forensic Sci 2000;45(1):237–239.

Sir:

The article of Wu et al. and the following discussion touched an extremely important point in the interpretation of analytical data, i.e., the limitations of existing techniques. Crouch and Wu stated very correctly in their response, that all analytical methods have limitations. However, their defense of single ion monitoring CI-MS as an ultimate identification tool in forensic toxicology may lead not only to misinterpretation of data, but also to some scientific stagnation. The authors stated that the condemnation of a time tested technique based on one example is "unwarranted." In fact, an example cited by Kidwell and Smith is only a peak of the iceberg. This is particularly true for illicit psychoactive phenethylamines, which very often possess the same molecular mass, but quite different structures, different pharmacokinetical properties and different legal classification. In the database "Structural Data of Compounds under Control" (1) ten phenethylamines are listed with molecular mass around 193, corresponding to MDMA. Six of them have a molecular formula $C_{11}H_{15}NO_2$ and exact molecular mass 193.24564. Among these drugs are: 2-3-MDMA, 3-4-MDMA, 2-3BDB, 3,4-BDB and two other compounds. Four compounds possess a formula $C_{12}H_{19}NO$, corresponding to the mass 193.28900. The differentiation of all these drugs is possible only through an appropriate fragmentation.

The statement of Crouch and Wu, that the condemnation of single ion CI-MS causes a precondemnation of recent innovations, like LC-API-MS or LC-API-MS/MS, seems to be not substantiated. The most important feature of LC-API-MS, irrespective of the kind of ionization source or of mass analyzer, is the possibility of fragmentation, known as collision induced dissociation (CID). CID is possible not only in MS/MS instruments, but also, as "in source CID" in less expensive LC-API-MS machines. From our experience we may say that LC-APCI-MS is a very powerful identification technique, due to the possibility of controlled fragmentation. In some cases, however, the fragmentation was not feasible in our conditions, like, i.e., for morphine or codeine. In these cases it is possible to support the positive result for morphine or codeine with simultaneous determination of metabolites: morphine-3-glucuronide, morphine-6-glucuronide and codeine-6-glucuronide. All these metabolites reveal a protonated molecular peak and parent drug ion as an aglycone. In all other cases, the identification of common drugs of abuse was based on the detection of at least one fragment additional to protonated molecular peak (2). Also in the case of determination of psychoactive phenethylamines with LC-APCI-MS (3) it was always possible to detect two or three characteristic ions (including protonated molecular peak). This made possible to differentiate MDEA from MBDB (molecular mass 207) or methamphetamine from phentermine (molecular mass 149). It must be mentioned that both pairs of drugs mentioned were not chromatographically separated.

The detection of a peak in SIM-CI-MS, which corresponds in retention behavior and molecular mass to a given substance, should be regarded as a hint of its identity, but certainly not as a positive and forensic defendable proof.

References

1. Rösner P, Junge TH, Stobbe S, Fritschi G. Structural data of compounds under control. Digilab Software, 24229 Scharnhagen, September 1999.
2. Bogusz MJ, Maier RD, Krüger KD, Kohls U. Determination of common drugs of abuse in body fluids using one isolation procedure and liquid chromatographic-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 1998;23:549–58.
3. Bogusz MJ, Krüger KD, Maier RD. Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol. In press.

Prof Dr.med M.J. Bogusz
Institute of Forensic Medicine
Aachen University of Technology
52057 Aachen, Germany

Authors' Response

Sir:

Thank you for the opportunity to respond to the comments of Dr. Bogusz. In our original article "Minimal Standards for the Performance and Interpretation of Toxicology Tests in Legal Proceeding" (JFS, May 1999), we stated that minimum standards are needed to ensure that the analysis for drugs, metabolites, and other toxins in biological specimens are properly performed. We presented a series of actual cases where (in our opinion) the criteria used for the identification, confirmation, quantitation of the drugs and interpretation of the data lacked scientific merit. We concluded the article with recommendations for laboratories to: (1) proactively establish policies for the acceptance, testing and the analysis of non-routine cases, (2) establish criteria for verifying and validating methods used in testing of routine and non-routine cases, (3) validate methods for each biological matrix that will be analyzed, (4) process controls, calibrators and other quality control materials with each analysis; these materials should be fortified into the matrix being analyzed and bracket the concentration detected/reported in the case sample(s), and (5) be conservative as forensic scientists in the reporting of analytical data and interpretations.

Our article prompted a communications challenging whether " . . . single ion monitoring may be acceptable if there are other corroborating analytic data to substantiate the analysis." In the letter by Smith and Kidwell (JFS, Jan 2000), they presented a case of possible misidentification of MDMA and N-methyltyramine. In that case, single ion monitoring CI-MS would have produced the same molecular ion (m/z 236) and GC retention times for both analytes. In the current letter by Dr. Bogusz, 10 phenethylamines were cited as having the poteintial to produce ambiguous results using single ion monitoring LC/MS. As we stated in our response to Drs. Kidwell and Smith, unique MS identification, (and particularly the specificity of CI-MS) depends on the extraction procedure, the derivative (if formed), reagent gas(es), source temperature, chemical structure of the analyte, the chromatographic separation, carrier gas, the scan function, whether positive or negative ions (or both) are detected, the experience of the analyst, and a host of related parameters.

Furthermore, Dr. Bogusz attributes the statement ". . . single ion monitoring CI-MS as an ultimate identification tool . . ." to us. That statement was not made in any of our communications. Nor, hopefully, was it implied. We did, however, make the following statements: "The use of single ion monitoring should be performed only under tightly controlled prevalidated conditions as it is not as definitive as full scan or selected ion analysis" (JFS, May 1999), and, "As scientists we should be cognizant of the fact that all methods have limitations" (JFS, Jan 2000).

We are well aware of the potential of LC-MS and LC-MS/MS (1–6) and have not written any comments that do not support these techniques. In fact, we stated ". . . as these (GC-MS/MS, LC-MS and LC-MS/MS) and other technologies develop, we urge forensic scientists to embrace them into their testing arsenal. . . ." (JFS, Jan 2000).

We hope that the comments that Dr. Bogusz and others have made will stimulate toxicologists to improve the quality of their work, as suggested in our original article.

References

1. Josephs JL, Crouch DJ, Spanbauer AC. Low level analysis of drugs in biological matrices by ESI-LC/MS. Application Report #249. pp 1–4. Finnigan-MAT, Oct, 1995.
2. Moody DE, Laycock JD, Spanbauer AC, Crouch DJ, Foltz RL. Determination of buprenorphine in human plasma by gas chromatography/positive ion chemical ionization mass spectrometry and liquid chromatography/tandem mass spectrometry. J Anal Toxicol 1997;21:1–9.
3. Crouch DJ. Forensic applications of LC/MS. LC/MS/MS analysis of alprazolam, haloperidol, morphine, and its glucuronide metabolites. Presented at the Society of Forensic Toxicologists. Salt Lake City, UT, 1997.
4. Crouch BI, Crouch DJ, Kinck K, Matsumura K, Rollins DE. Cardiac arrest, seizures and one death associated with benzonatate ingestion. Clin Tox 1998;36;7:713–8.
5. Crouch DJ, Rollins DE, Canfield DV, Andrenyak DM, Schulties JE. Quantitation of alprazolam and alpha-hydroxyalprazolam in human plasma using liquid chromatography electrospray ionization MS/MS. J Anal Toxicol 1999;23;6:479–85.
6. Slawson MH, Crouch DJ, Andrenyak DM, Rollins DE, Lu JK, Bailey PL. Determination of morphine, morphine-3-glucuronide and morphine-6-glucuronide in plasma following intravenous and intrathecal morphine administration utilizing HPLC with electrospray tandem mass spectrometry. J Anal Toxicol 1999;23:468–73.

Dennis J. Crouch, B.S., MBA
Interim Director, Center for Human Toxicology
Research Assistant Professor, Phamracology and Toxicology
University of Utah
Salt Lake City, UT 84112

Alan H.B.Wu, Ph.D.
Director, Clinical Chemistry and Toxicology
Hartford Hospital
80 Seymour St.
Hartford, CT 06102

Promega Corporation Reveals Primer Sequences in Its Testing Kits

Sir:

Recently, a Vermont District Court ruled that, "the Profiler Plus™ and PowerPlex™ amplification kits manufactured by Perkin-Elmer/Applied Biosystems and Promega, respectively, are not admissible under the relaxed standard of Daubert" (1). This is the first time that the DNA typing results using a kit manufactured by Promega has been ruled inadmissible in a court of law. One of the major concerns expressed by this court was that the primer pair DNA sequences had not been made public and could not, therefore, be subjected to peer review. The judge cited the guidelines established by the DNA Advisory Board in his ruling.

Even though it is clear to the scientific community that knowledge of the primer sequence, in and of itself, is not scientifically relevant to obtaining reliable and accurate DNA typing results, we believe the Vermont court's concerns pose a serious problem for the forensic community. Specifically, unless the DNA sequences for the primer pairs used in our kits are made public, we are quite concerned that an unacceptable amount of time of forensic scientists will be spent testifying in courts to defend the use of commercially available DNA testing kits. As a result, the Promega Corporation decided in May of this year to disclose our primer pair sequences to the forensic community. Even though we see the primer pair sequence information as a critical and proprietary element of the intellectual property of our DNA testing systems, the need of the forensic community outweighs our own interests. Promega's action is not simply to acquiesce to every defense request. Our intent is to assist forensic scientists, who have come to depend on commercially available DNA typing kits in their work, to provide independent scientific data to corroborate events at a crime scene to law enforcement agencies.

In this letter, we have included the DNA sequences for the following kits sold by Promega Corporation: PowerPlex™ 1.1, PowerPlex™ 1.2, and FFFL (Tables 1–4). The validation of Power-

TABLE 1—*PowerPlex™1.1 primer sequences.*

Locus	Oligonucleotides Sequences	5' end
CS1PO Primer Pair	AAC CTG AGT CTG CCA AGG ACT AGC	TMR
	TTC CAC ACA CCA CTG GCC ATC TTC	OH
TPOX Primer Pair	ACT GGC ACA GAA CAG GCA CTT AGG	OH
	GGA GGA ACT GGG AAC CAC ACA GGT TA	TMR
TH01 Primer Pair	ATT CAA AGG GTA TCT GGG CTC TGG	OH
	GTG GGC TGA AAA GCT CCC GAT TAT	TMR
vWA Primer Pair	GCC CTA GTG GAT GAT AAG AAT AAT CAG TAT GTG	OH
	GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG	TMR
D16S539 Primer Pair	GGG GGT CTA AGA GCT TGT AAA AAG	FL
	GTT TGT GTG TGC ATC TGT AAG CAT	OH
D7S820 Primer Pair	ATG TTG GTC AGG CTG ACT ATG	FL
	GAT TCC ACA TTT ATC CTC ATT GAC	OH
D13S317 Primer Pair	ACA GAA GTC TGG GAT GTG GA	OH
	GCC CAA AAA GAC AGA CAG AA	FL
D5S818 Primer Pair	GGT GAT TTT CCT CTT TGG TAT CC	OH
	AGC CAC AGT TTA CAA CAT TTG TAT CT	FL

TABLE 2—*Amelogenin primer pair sequences (for coamplification with PowerPlex™1.1).*

Locus	Oligonucleotide Sequences	5' end
Amelogenin Primer Pair	ACC TCA TCC TGG GCA CCC TGG	OH
	AGG CTT GAG GCC AAC CAT CAG	TMR

TABLE 3—*F13A01, FESFPS, F13B, LPL (FFFL) primer pair sequences.*

Locus	Oligonucleotide Sequences	5' end
F13AO1 Primer Pair	GAG GTT GCA CTC CAG CCT TTG CAA	FL
	TTC CTG AAT CAT CCC AGA GCC ACA	OH
FESFPS Primer Pair	GCT GTT AAT TCA TGT AGG GAA GGC	FL
	GTA GTC CCA GCT ACT TGG CTA CTC	OH
F13B Primer Pair	TGA GGT GGT GTA CTA CCA TA	FL
	GAT CAT GCC ATT GCA CTC TA	OH
LPL Primer Pair	CTG ACC AAG GAT AGT GGG ATA TAG	FL
	GGT AAC TGA GCG AGA CTG TGT CT	OH

TABLE 4—*PowerPlex™1.2 primer pair sequences.*

Locus	Oligonucleotides Sequences	5' end
CSF1PO Primer Pair	AAC CTG AGT CTG CCA AGG ACT AGC	TMR
	TTC CAC ACA CCA CTG GCC ATC TTC	OH
TPOX Primer Pair	ACT GGC ACA GAA CAG GCA CTT AGG	OH
	GGA GGA ACT GGG AAC CAC ACA GGT TA	TMR
Amelogenin Primer Pair	ACC TCA TCC TGG GCA CCC TGG TT	OH
	AGG CTT GAG GCC AAC CAT CAG	TMR
TH01 Primer Pair	ATT CAA AGG GTA TCT GGG CTC TGG	OH
	GTG GGC TGA AAA GCT CCC GAT TAT	TMR
vWA Primer Pair	GCC CTA GTG GAT GAT AAG AAT AAT CAG TAT GTG	OH
	GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG	TMR
D16S539 Prime Pair	GGG GGT CTA AGA GCT TGT AAA AAG	OH
	GGT TGT GTG TGC ATC TGT AAG CAT GTA TC	FL
D7S820 Primer Pair	ATG TTG GTC AGG CTG ACT ATG	FL
	GAT TCC ACA TTT ATC CTC ATT GAC	OH
D13S317 Primer Pair	ACA GAA GTC TGG GAT GTG GAG GA	OH
	GGC AGC CCA AAA AGA CAG A	FL
D5S818 Primer Pair	GGT GAT TTT CCT CTT TGG TAT CC	OH
	AGC CAC AGT TTA CAA CAT TTG TAT CT	FL

Plex™ 1.1 and FFFL was published earlier in this journal (2), and we hope that the community will view this letter as an addendum to that publication. In the near future, we, and the forensic labs working with us, will be publishing articles describing the validation of two more DNA typing systems, PowerPlex™ 16 and PowerPlex™ 2.1. These publications will include the primer pair sequences for these kits. In the meantime, Promega has and will continue to provide this sequence information at appropriate scientific meetings.

We have also modified our validation efforts in a number of ways to aid the forensic community's efforts to address anticipated court challenges. Specifically, our validation efforts on both PowerPlex™ 2.1 and PowerPlex™ 16 systems include wide representation of laboratories throughout the United States with over 35 laboratories participating in the two efforts. The results of these joint projects will demonstrate that these systems have been widely tested and validated as part of their introduction into the field. We expect that this will reduce the burden of the forensic community in their defense of the use of these systems in a court setting. We are convinced that the use of DNA testing in a forensic setting benefits both defense and prosecuting attorneys in assessing the facts of the case.

References:

1. *State of Vermont* v. *Michael Pfenning,* District Court, Grand Isle County, Case No. 57-4-96 GiCr.
2. Micka, KA, et al., TWGDAM validation of a nine-locus and a four-locus fluorescent STR Multiplex System. J Forensic Sci 1999;44(6).

Arni Masibay, Ph.D.
Regional Technical Specialist
Promega Corporation

Thomas J. Mozer, Ph.D.
Director, Human Identification Systems,
Promega Corporation

Cindy Sprecher,
R&D Project Manager,
Promega Corporation