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The Second Seven Years of the FAA's Postmortem Forensic Toxicology Proficiency-Testing Program

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16. Abstract

For aircraft accident investigations, samples from pilot fatalities are analyzed at the Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) for the presence of combustion gases, alcohols/volatiles, and drugs. Throughout this forensic toxicological process, a high degree of quality control/quality assurance (QC/QA) is maintained, and quality improvement is continuously pursued. Under this philosophy, CAMI started a quarterly forensic toxicology proficiency-testing (PT) program in July 1991 for the analysis of postmortem specimens. In continuation of the first 7 years of the CAMI PT findings reported earlier, PT findings of the next 7 years (July 1998–April 2005) are summarized herein. During this period, 28 PT challenge survey samples (12 urine, 9 blood, and 7 tissue homogenate) with/without alcohols/volatiles, drugs, drug metabolites, and/or putrefactive amine(s) were submitted to an average of 31 participating laboratories, of which an average of 25 participants returned their result sheets—that is, 53–96% (mean = 82%). The number of respondents was dependent upon the complexity of the sample matrix, the number and types of analytes in the sample, and the associated analytical chemistry/toxicology. For example, ethanol/methanol/volatiles in urine were correctly quantitated by a higher number of participants than those for amphetamine/methamphetamine and cannabinoid levels in blood and tissues. Methods employed ranged from immunoassays to gas chromatography-mass spectrometry/high-performance liquid chromatography. Analytes in survey samples were correctly identified and quantitated by a large number of participants, but some false positives of concern were reported, as some of them were abused drugs. Some of the false positives would have been avoided by not reporting those drugs solely based upon presumptive analyses. Their presence should have been confirmed, authenticated, and, if possible, quantitated by other analytical methods, which should have been based upon different analytical principles than those used during presumptive analyses. It is anticipated that the FAA's PT program would continue to serve as a tool to effectively allow its own toxicology laboratory and other participating laboratories for professional and technical maintenance and advancement on a voluntary, interlaboratory, and self-evaluative basis. Furthermore, this PT program will continue to provide service to the forensic toxicology scientific community through this important part of the QC/QA for the laboratory accreditation to withstand professional and judicial scrutiny of analytical results.

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The Second Seven Years of the FAA's Postmortem Forensic Toxicology Proficiency-Testing Program

INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) conducts toxicological evaluation of postmortem biological samples collected from fatally injured pilots involved in civil aircraft accidents (1). The submitted samples are analyzed for the presence of primary combustion gases, alcohols/volatiles, and drugs (2). Throughout the entire evaluation process, a high degree of quality control/quality assurance (QC/QA) is maintained, and quality improvement is continuously pursued (3-6). The participation of laboratories in external proficiency-testing (PT) programs is considered an integral part of QC/QA of a laboratory and its accreditation (4,7,8).

In view of the quality enhancement, CAMI developed, implemented, and sponsored a PT program, effective July 1991 (9,10). This PT program was designed for the analysis of postmortem specimens, which closely represented the types and quality of specimens received from aircraft accident pilot fatalities and from death cases encountered in medical examiner and coroner systems. Details of this program have been published earlier (9,10). Briefly, this quarterly PT program is designed to professionally develop and maintain technical currency on a voluntary, interlaboratory, and self-evaluation basis and to quantifiably assess methods in the absence and presence of interfering substances. Findings of the first 7 years (July 1991–April 1998) of the CAMI PT surveys were summarized in these 2 publications. In continuation, CAMI PT findings of the next 7-year (July 1998–April 2005) surveys are described herein.

MATERIALS AND METHODS

Materials

Drug-free human urine was obtained from a commercial source; human whole blood was supplied by a local blood bank. Animal tissues were purchased from local meat markets. Drugs, metabolites, and chemicals/substances were obtained from commercial sources.

Survey Samples

Urine did not require any initial treatment prior to its use for the preparation of survey samples, though sodium fluoride was added to blood obtained from the local blood bank to achieve a 1% solution. Animal tissues were weighed, cut into small pieces, and homogenized in deionized water. In urine, blood, and homogenates, measured amounts of analytes, putrefactive bases (ß-phenethylamine, tryptamine, and/or tyramine), and/or other toxicologically relevant substances were added, mixed, and allowed to equilibrate for at least 24 hours prior to the distribution of PT survey samples to the participating laboratories (Table I). The final tissue homogenate mixture contained 1 part of tissue to 2 parts of water by weight—that is, 3 g of homogenate contained 1 g of tissue. With some survey samples, putrefaction processes were initiated by keeping those samples at ambient temperature for selected periods. Stock solutions of analytes were prepared in appropriate solvents. In some samples, no analytes of interest were added. Such samples were considered as "Negatives."

Human urine and blood were screened for the presence of alcohols/volatiles and commonly encountered drugs prior to their use in the preparation of PT survey samples. The methods for the screening might not rule out the presence of those drugs—if they were present in amounts below the detectable limits of the screening methods. Other drugs that could not be screened by the employed methods might also be present in the survey samples. Animal tissue homogenates were not screened for the presence of commonly used drugs in humans, but chemical substances of veterinary medical practices might be present in such survey samples.

Survey Sample Distribution and Result Summaries

Urine, blood, and homogenate survey samples were shipped in suitable containers in appropriate amounts with frozen gel bags in an insulated box by an air courier service for next-day delivery to participating laboratories (9,10). The sample shipment occurred in the months of January, April, July, and October on a yearly cycle —that is, 4 PT survey samples were distributed in a year. To the CAMI laboratory, the PT survey samples were hand delivered on the next day of the shipment of samples to other participants.

All participants were requested to return analytical report sheets of PT surveys by due dates, even if their laboratory did not routinely analyze a particular analyte in a particular specimen type. Unless all analytical report

Table I. PT Survey Sample Description and Participants' Analytical Responses

Survey Sample No.	Specimen Types	Analytes' Weighed-in Concentrations	Respondents' Analyses Details			Participants/ Respondents (% Responded)
			Mean Concentrations (SD_ns; if n ≥ 5)*	% Values Within 2 SD_ns	Qualitative (only)/ Quantitative	
1	Bovine brain†	No substance added (negative)	---	---	---	31/22 (71)
2	Human urine	Salicylic acid (100 µg/mL)	---‡	---	3/4	31/24 (77)
		Theophylline (50 µg/mL)	---‡	---	6/4	
3	Human blood	Atropine (517 ng/mL)	---‡	---	8/3	31/24 (77)
		Digoxin (27 ng/mL)	---	---	0/0	
		Ethanol (70 mg/dL)	56 (4)	100	0/20	
4	Human blood	Alprazolam (50 ng/mL)	---‡	---	5/4	31/25 (81)
		α-Hydroxyalprazolam (10 ng/mL)	---	---	2/0	
		Ethanol (70 mg/dL)	67 (6)	100	0/22	
		Methanol (8 mg/dL)	---‡	---	1/4	
		Methylphenidate (1,170 ng/mL)	738 (147; n = 6)	100	6/12	
5	Bovine brain†	No substance added (negative)	---	---	---	31/23 (74)
6	Porcine liver†	Ethanol (81 mg/hg)	81 (20)	100	1/7	34/18 (53)
		Methanol (27 mg/hg)	42	---	0/1	
		ß-Phenethylamine (11 µg/g)	---	---	---	
		11-Hydroxy-Δ^9-tetra-hydrocannabinol (51 ng/g)	---	---	0/0	
		11-nor-Δ^9-Tetrahydrocannabinol-9-carboxylic acid (501 ng/g)	---‡	---	0/2	
		Δ^9-Tetrahydrocannabinol (300 ng/g)	---	---	1/0	
7	Human urine	Bupropion (2 µg/mL)	---‡	---	13/3	33/26 (79)
		Bupropion metabolite (3 µg/mL)	4	---	6/1	
		Paroxetine (2 µg/mL)	---‡	---	8/3	
8	Human urine	No substance added (negative)	---	---	---	33/29 (88)

9	Human blood	No substance added (negative)	---		---	---	33/27 (82)
10	Human blood	Benzoylecgonine (98 ng/mL)		116 (16)	100	3/11	33/29 (88)
		Cocaine (203 ng/mL)		183 (49)	100	10/15	
		Methanol (13 mg/dL)		12 (1; n = 5)	100	1/6	
		Phencyclidine (97 ng/mL)		86 (15; n = 14)	100	9/16	
		Δ^9-Tetrahydrocannabinol (50 ng/mL)		44; 50	---	0/2	
11	Human urine	Ethanol (16 mg/dL)		14 (3)	100	0/8	33/26 (79)
		Oxazepam (212 ng/mL)		247 (60; n = 5)	100	7/7	
12	Human blood	Acetaminophen (16 µg/mL)		15 (3)	100	1/6	34/25 (74)
		Ethanol (93 mg/dL)		76 (6)	94	0/18	
		Fluoxetine (111 ng/mL)		101 (21; n = 6)	100	6/7	
		Norfluoxetine (144 ng/mL)	---‡		---	3/4	
13	Porcine liver†	ß-Phenethylamine (15 µg/g)	---		---	---	34/23 (68)
		Tryptamine (15 µg/g)					
14	Human urine	Cimetidine (150 µg/mL)		38	---	4/1	34/26 (76)
		Desmethylsertraline (25 µg/mL)	---‡		---	13/5	
		Sertraline (20 µg/mL)	---‡		---	19/6	
15	Human urine	Diphenhydramine (2 µg/mL)	---‡		---	14/5	33/29 (88)
		Oxycodone (12 µg/mL)		12 (3; n = 6)	100	17/7	
16	Bovine liver†	No substance added (negative)	---		---	---	29/23 (79)
17	Human blood	*d*-Amphetamine (10 ng/mL)		10; 10; 10	---	2/3	29/25 (86)
		l-Methamphetamine (177 ng/mL)		167 (15)	90	1/10	
		ß-Phenethylamine (10 µg/mL)	---		---	---	
18	Human urine	Atenolol (100 ng/mL)	---		---	0/0	28/25 (89)
		Methanol (8 mg/dL)	---		---	0/0	
19	Bovine liver†,§	Hydrocodone (3 µg/g)	---‡		---	4/8	28/22 (79)
		Morphine (165 ng/g)	---‡		---	2/8	
20	Human urine	Chloroquine (19 µg/mL)		21 (4; n = 5)	100	15/6	28/27 (96)
		Quinidine (60 µg/mL)		57 (4; n = 5)	100	11/6	

21	Human urine	Ethanol (103 mg/dL)	102 (5; n = 14)	93	0/15	29/25 (86)
		Methanol (30 mg/dL)	30 (1)	100	3/7	
22	Human blood	Desipramine (345 ng/mL)	278 (66; n = 12)	100	9/13	29/26 (90)
		Imipramine (430 ng/mL)	400 (75)	93	8/14	
		ß-Phenethylamine (12 µg/mL)	---	---	---	
		Tryptamine (6 µg/mL)	---	---	---	
		Tyramine (6 µg/mL)	---	---	---	
23	Human urine	Acetone (25 mg/dL)	23 (3)	93	2/15	28/25 (89)
		Ethanol (77 mg/dL)	72 (5)	89	0/19	
		Isopropanol (74 mg/dL)	71 (3)	93	2/15	
		Methanol (52 mg/dL)	48 (5)	93	2/14	
24	Human urine	No substance added (negative)	---	---	---	28/26 (93)
25	Human blood	Carbamazepine (10 g/mL)	9 (2)	91	8/11	28/23 (82)
		Ethanol (79 mg/dL)	74 (5)	95	0/22	
		Phenobarbital (8 µg/mL)	8 (2; n = 11)	91	9/12	
26	Bovine liver[†,((]	No substance added (negative)	---	---	---	28/24 (86)
27	Human blood[((]	Acetaminophen (10 µg/mL)	10 (2; n = 9)	100	2/10	28/24 (86)
		Ethanol (158 mg/dl)	148 (9)	95	0/21	
		Ibuprofen (22 µg/mL)	12 (2)	100	5/5	
28	Human urine	No substance added (negative)	---	---	---	28/27 (96)

[*]Concentration units are the same, as are listed in the corresponding rows of the table's preceding column (No. 3). No statistical analyses were performed in those analyte analysis values when there were large variations in quantitative values and/or limited quantitative values of 4 or less. Associated details are given in the Materials and Methods section.

[†]Homogenates of solid tissue types were prepared in deionized water in the proportion of 1 part tissue to 2 parts deionized water by weight—that is, 3 g of homogenate contained 1 g of tissue. The quantitative values are expressed as the concentrations in the tissues rather than in the homogenates.

[‡]There were large variations in quantitative values. No meaningful statistical analysis could be finalized in spite of the one-by-one elimination of the numerical values and the determinations of means and SD_ns with the SD_n value ≤ 25% of the respective mean and with the "n" value ≥ 5. Therefore, no quantitative values are mentioned herein in the interest of space in the table. Related details are given in the Materials and Methods section.

[§]The result summary report of this survey sample was amended due to a miscalculation of the amount of morphine sulfate added as free base in the preparation. Initially, it was calculated as 1 molecule of morphine per morphine sulfate, rather than 2 molecules of morphine.

[((]The specimens were putrefied by keeping them at room temperature for 2 days prior to their distribution to participants.

sheets were returned, it could not be verified that all participating laboratories received and responded to a particular PT sample. In addition to reporting qualitative and quantitative results, those analytical report respondents had an option to defer a survey sample analysis by choosing an appropriate box on the report sheet—that is, "do not perform analysis on this specimen type" or "choose not to perform analysis due to other reasons." Such deferments within the report respondents were considered as analysis deferments. Within a 4-week period after the last date of the report submission, a summary of the results of PT surveys were prepared and sent to the participating laboratories (9,10).

Statistical Calculations

The mean and standard deviation of quantitative analytical values for each analyte were calculated by using Texas Instruments TI-60 Advanced Scientific Calculator (Texas Instruments Professional TI-60 Guide Book 1986, Lubbock, TX) or by using Microsoft® Office Excel 2003 (Redmond, WA). The standard deviation calculation was based upon the entire population given as argument—that is, data taken from every member of a population—and is abbreviated herein as SD_n, where "n" is the number of the analytical values for a particular analyte.

For the purpose of the PT survey result summarization, wherein 5 or more quantitative values were received from the participants for an analyte, the mean of analytical values and SD_n were calculated. If the SD_n was found to be > 25% of the mean, an "out-of-range" value was identified for the purpose of the result summarization. An "out-of-range" value was defined as the one which had the largest absolute difference from the mean value. This "out-of-range" numerical value was identified and removed from the tabulated values. Subsequently, the new mean and SD_n with the new "n" numbers—that is, "n – 1" numbers—were calculated. If the new SD_n was < 25% of the mean, then the mean and SD_n values were included in the table; otherwise, a new mean and SD_n were calculated after removing another "out-of-range" value with the largest absolute difference from the new mean. This process of one-by-one eliminations of values and calculations of means was continued until an SD_n value of < 25% of the mean was obtained. On few occasions, in spite of the elimination of values, it was not possible to obtain an SD_n value that was < 25% of the mean. If only 4 or less quantitative analytical values were left after the one-by-one elimination process or if only 4 or less quantitative values were obtained from the analytical reports of the participants, then no mean and SD_n were determined. In summary, no statistical analyses were reported in those situations wherein there were large variations in quantitative values and/or limited quantitative values (≤ 4). The quantitative values with large variations are not included in the table in the interest of space.

The report respondents and analysis deferments for various sample types were analyzed at $\alpha = 0.05$ using analysis of variance and Duncan's multiple-range test for statistical pair-wise differences between the groups (Fig. 1). The statistical software package used for the analysis of variance and the multiple-range test was SAS, version 9.1 (SAS Institute, Inc., Cary, NC). The report respondents were those participants who returned the analytical report sheets, and the analysis deferments were those participants who also deferred the analysis by marking an appropriate box on the analytical report sheet.

RESULTS

Throughout the second 7 years of the CAMI PT survey, a total of 28 samples were submitted to 28–34 (mean = 31; SD_n = 2) participating laboratories. However, not all of the participants returned the analytical report sheets of a particular survey. Only 18–29 (mean = 25; SD_n = 2) participants returned their results—that is, 53–96% (mean = 82; SD_n = 9) of the total participants. The PT survey consisted of 12 urine, 9 blood, and 7 tissue (2 brain and 5 liver) homogenate specimens. No drugs were added to 9 of the 28 survey samples, while 2 analytes were added to 9 samples, 3 to 5, 4 to 2, and 5 to 3 samples (Table I). The analytes added to the survey samples covered the whole spectrum of volatiles and drugs. The former analyte category contained acetone, ethanol, isopropanol, and methanol. The latter consisted of acidic, neutral, and basic drugs, covering prescription and nonprescription drugs and controlled substances of Schedules I–V (11). Analytes were added in subtherapeutic-to-therapeutic or subtoxic-to-toxic concentrations reported in the literature (12-18). PT survey details, covering mean concentrations with SD_ns and percentage of values falling within 2 SD_n values, are given in Table I. As an average, 97% (89–100%; SD_n = 4) of the analytical values fell within 2 SD_ns; 78% of the means of the analyte quantitative values were within 20% of their weighed-in amounts in the survey samples. There were some obvious clerical, transcription, or typographical errors in reported units and/or decimal places. Such numerical values were not included in the statistical calculations. Examples of these types of errors were: 0.08 mg/L of methylphenidate instead of 0.8 mg/L; 0.10% of methanol in place of 0.010%; 0.209 g/dL of ethanol instead of 0.104 g/dL; and11.6 mg/dL of acetaminophen in place of 11.6 µg/mL.

The number of analytical report respondents of a survey was dependent upon the complexity of the sample matrix characteristics (blood, urine, or homogenate; putrefied or non-putrefied), number and types of analytes (alcohols,

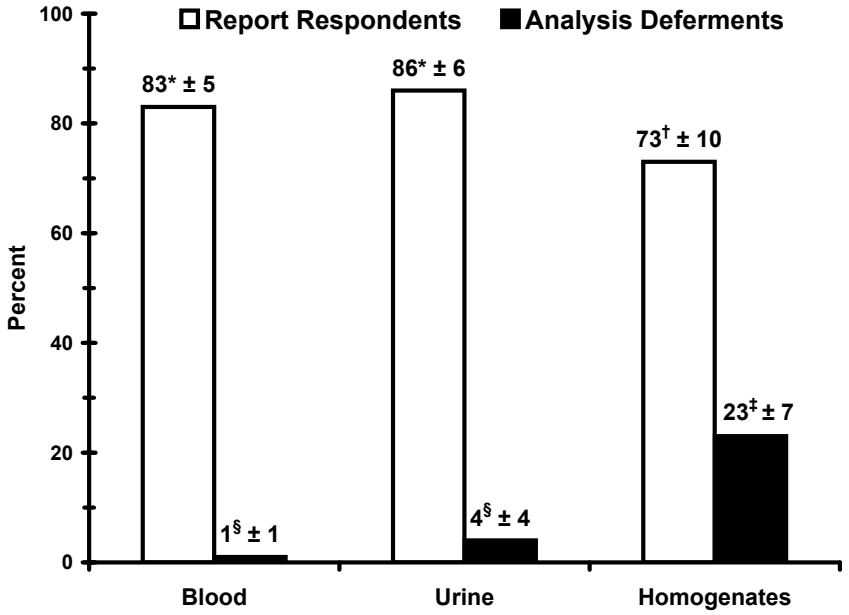

Figure 1. Analytical report respondents and analysis deferments (within the report respondents) for PT survey sample types. Histograms represent percent means of the respondents or deferments for blood (n = 9), urine (n = 12), and tissue homogenates (n = 7); numbers after "±" are corresponding SD_ns. The analysis of variance of the 6 groups indicated a significant difference in the means at $p < 0.0001$. Bars marked with the same symbol indicate that those values are not significantly different from each other, but the values designated by the different symbol are different at $\alpha = 0.05$.

11-hydroxy-Δ^9-tetrahydrocannabinol, opiates, and/or benzodiazepines), and associated analytical chemistry/toxicology. Volatiles in urine were correctly quantitated by the majority of participants, whereas amphetamine/methamphetamine and cannabinoid levels in blood and tissues were reported by a considerably lower number of participants. Methods employed ranged from immunoassays to gas chromatography-mass spectrometry/high performance liquid chromatography. The analytical report sheets of blood and urine survey samples were returned by 83% ($SD_n = 5$; n = 9) and 86% ($SD_n = 6$; n = 12) of the participants, respectively (Fig. 1), whereas, such response was 73% ($SD_n = 7$; n = 7) with homogenates. The response with homogenates in comparison to that with urine or blood was statistically significant ($\alpha = 0.05$). Within the analytical report respondents, the deferment of analysis was significantly high (23%; $SD_n = 7$; $\alpha = 0.05$) with homogenates in comparison to that with blood (1%; $SD_n = 1$) or urine (4%; $SD_n = 4$). Two such examples are: (i) the report was returned by only 53% of the participants of which 28% deferred the analysis for a porcine liver homogenate spiked with alcohols, cannabinoids, and a putrefactive amine and (ii) the report was returned by 86% of the participants, but 33% of those deferred the analysis of a negative bovine liver homogenate.

False positives of concern were reported in 8 out of the 28 surveys (Table II). The number of laboratory-reported positives was 1 for each of the 7 surveys, but 2 laboratories reported amphetamines or amphetamine class in 1 survey. Five of the 7 positive analytes were benzoylecgonine, flunitrazepam, phenylpropanolamine, lysergic acid diethylamide, and quinine. The respective specimen types (intended analytes) were bovine brain homogenate (negative), human blood (alprazolam, α-hydroxyalprazolam, ethanol, methanol, and methylphenidate), porcine liver homogenate (ethanol, methanol, 11-hydroxy-Δ^9-tetrahydrocannabinol, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid, and Δ^9-tetrahydrocannabinol), and human urine (2 urine surveys: one contained cimetidine, desmethylsertraline, and sertraline, while the other chloroquine and quinidine). The 3 remaining survey samples were reported to qualitatively contain amphetamine, methamphetamine, or amphetamine/amphetamine class drugs. Two of these 3 samples—porcine liver homogenate and human blood—were spiked with ß-phenethylamine, tryptamine, and/or tyramine. The last survey sample was not spiked with any putrefactive amine, but 1 laboratory reported the presence of methamphetamine by using a gas chromatography-mass spectrometry method. The survey sample porcine liver homogenate, in which phenylpropanolamine was reported, was also spiked with ß-phenethylamine.

Table II. Survey Sample Types/Analytes and False Positives of Concern Reported by Laboratories

Survey Sample No.*	Specimen Types	Analytes' Weighed-in Concentrations	False Positives of Concern (No. of Laboratories)	Method/Techniques Used	Qualitative or Quantitative Analysis
1	Bovine brain†	No substance added (negative)	Benzoylecgonine (1)	Fluorescence polarization immunoassay	2.4 µg/g
4	Human blood	Alprazolam (50 ng/mL) α-Hydroxyalprazolam (10 ng/mL) Ethanol (70 mg/dL) Methanol (8 mg/dL) Methylphenidate (1,170 ng/mL)	Flunitrazepam (1)	Enzyme-linked immuno-sorbent assay	Qualitative
6	Porcine liver†	Ethanol (81 mg/hg) Methanol (27 mg/hg) ß-Phenethylamine (11 µg/g) 11-Hydroxy-Δ^9-tetra-hydrocannabinol (51 ng/g) 11-nor-Δ^9-Tetrahydrocannabinol-9-carboxylic acid (501 ng/g) Δ^9-Tetrahydrocannabinol (300 ng/g)	Phenylpropanolamine (1)	Enzyme immunoassay; gas chromatography-mass spectrometry	Qualitative
13	Porcine liver†	ß-Phenethylamine (15 µg/g) Tryptamine (15 µg/g)	Amphetamine/ methamphetamine (1)	Fluorescence polarization immunoassay	Qualitative
14	Human urine	Cimetidine (150 µg/mL) Desmethylsertraline (25 µg/mL) Sertraline (20 µg/mL)	Lysergic acid diethylamide (1)	Enzyme immunoassay	Qualitative

20	Human urine	Chloroquine (19 µg/mL) Quinidine (60 µg/mL)	Quinine (1)	Gas chromatography-mass spectrometry; high-performance liquid chromatography; thin layer chromatography	5 ng/mL
21	Human urine	Ethanol (103 mg/dL) Methanol (30 mg/dL)	Methamphetamine (1)	Gas chromatography-mass spectrometry	Qualitative
22	Human blood	Desipramine (345 ng/mL) Imipramine (430 ng/mL) ß-Phenethylamine (12 µg/mL) Tryptamine (6 µg/mL) Tyramine (6 µg/mL)	Amphetamines (1) Amphetamine class (1)	Enzyme immunoassay Enzyme immunoassay	500 ng/mL Qualitative

*Numbers in this column refer to those in Table I, along with the respective sample types and weighed-in concentrations of analytes. Analyses details and numbers of participants/respondents are given in Table I.

†Homogenates of solid tissue types were prepared in deionized water in the proportion of 1 part tissue to 2 parts deionized water by weight—that is, 3 g of homogenate contained 1 g of tissue. The quantitative values are expressed as the concentrations in the tissues rather than in the homogenates.

DISCUSSION

Since 1991, the FAA's PT program has been serving as an instrument for the FAA's own toxicology laboratory and other participating laboratories to evaluate their proficiency for forensic toxicology analysis. Having a broad national geographic coverage, these participants represent a wide spectrum of the nation's postmortem toxicology laboratory system and currently do not pay to participate in the PT program. Although this program does not fulfill any regulatory requirements, it has been effectively used by toxicology laboratories for their professional and technical maintenance and advancement on a voluntary, interlaboratory, and self-evaluative basis (9,10). The program has been serving as a tool for the assessment of analytical methods in the presence and absence of postmortem interfering substances and a means for the participating laboratories to mutually share scientific and technical information that reflects the proficiency in postmortem toxicological practices. This PT survey has been a valuable program that (i) entails the analysis of postmortem samples of complex matrixes, such as putrefied blood and other tissues, thus requiring specialized analytical approaches and (ii) successfully fulfills the requirement of the QC/QA component of the accreditation of laboratories (7-10).

As was observed during the first 7 years (9,10), not all participants returned their analytical report sheets, and because anonymity of the participants and of their results is strictly maintained, it was not possible to find out which laboratories did not return their report sheets. The number of qualitative and quantitative analytical result responses was dependent upon the complexity, condition, and characteristics of the sample matrixes, number and types of analytes present in the samples, and associated complexity of analytical chemistry/toxicology, including the stability of the analytes in a particular biological matrix and their common usage and related medicolegal implications.

Quantitative values were in remarkably good agreement with the respective target concentrations. In the majority of the cases, the quantitative values were within 2 SD_n of the means of the reported values, excluding any "unacceptable" values, such as values with decimal errors or wrong units/amounts, and/or not within 20% of the weighed-in amounts of the analytes. One aspect of the quantitation of basic drugs is worth emphasizing—that is, the nature of their salts used for the preparation of their controls, calibrator solutions, and associated calibration curves. Monobasic, dibasic, or tribasic nature of the drug salt should be taken into account when calculating the amount of the basic drug present in the sample by using the correct molecular weight of the drug salt and, thus, by knowing the number of drug molecules that would dissociate from each molecule of the drug salt. An example is an inadvertent miscalculation of the amount of morphine sulfate used for morphine in a survey sample, wherein the initial calculation was as 1 molecule, rather than 2 molecules, of morphine per 1 molecule of morphine sulfate. Because of this calculating error, the summary of results was amended and the summary was reissued.

Although survey sample matrixes were screened for the presence of commonly used drugs or they were of animal origin, the occasional presence of some analytes that were not added in a particular sample should not be construed as false positives. However, their presence could be of concern, particularly if they were controlled substances. Those analytes might have been genuinely present in the matrix used for the preparation of a PT challenge. As is true with any screening method, the method used for the screening might not necessarily be in a position to determine the presence of all possible drugs, if they were present in amounts below the detectable limits of the screening assays. Veterinary drugs might be present in the animal tissue homogenate samples, and macromolecules of animal origin in the tissue homogenates might interfere with antibody-based screening methods, thereby leading to false positives or negatives. However, it is being suggested that such positive findings should be supported by the analytical results obtained following the laboratory's standard operating procedures. The genuine presence of those analytes could also be deduced by the analytical results of other participants tabulated in the analytical summary reports. If several participants reported the particular analyte(s), then that analyte(s) could be concluded as true positive(s), otherwise viewed as an isolated incidence (9,10).

The reporting of caffeine, theobromine, theophylline, and nicotine should not be considered as false positives. Their presence was likely due to the consumption of caffeinated beverage, active/passive inhalation of cigarette smoke, or chewing of tobacco by the donors of the biological matrixes. These analytes were not added in the survey samples and may not necessarily be considered as drugs of use. The presence of ethanol or other alcohols/volatiles in samples not fortified by these analytes might have been associated with their production by microorganisms. Such production would be more prevalent if the samples did not have preservatives and were exposed to uncontrolled temperature conditions for various lengths of time. Reporting of ß-phenethylamine, tryptamine, and/or tyramine could not be of significance as these analytes are endogenous amines or putrefactive bases.

The majority of false positives of concern were reported based upon presumptive analyses (screening assays). Although the presence of phenylpropanolamine, methamphetamine, and quinine were generally demonstrated by immunoassays and gas chromatography-mass spectrometry methods, other false positives were found by only immunoassays. The reporting of phenylpropanolamine and amphetamine/methamphetamine might have been attributed to the presence of β-phenethylamine, a putrefactive interfering amine with these groups of structurally similar drugs (19-21). Such drugs should not have been reported solely based upon presumptive analyses. Their presence should have been confirmed, authenticated, and, if possible, quantitated by another analytical method that is based upon a different analytical principle than that used during the presumptive analysis.

With the analytical results, participants also provided the methods used for the analysis from a list of possible methods. This information was for the utilization by other laboratories to understand the analytical approaches taken. To further improve the PT program and associated analytical processes, the participants are now requested to choose from a list of types of extraction procedures they used during a survey sample analysis. Such information will be incorporated in the third segment of the FAA's PT program summarization, with a view that it will further sharpen the analytical efficiency of the participating laboratories. It is anticipated that the FAA's PT program will continue to provide service to the forensic toxicology scientific community through this important part of the QC/QA in the laboratory accreditation process to withstand professional and judicial scrutiny of analytical results.

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