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FAA Postmortem Forensic Toxicology Proficiency Testing Program: The Final Seven Years

Philip M. Kemp
Kristi J. Craft
Kristi Thompson

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Final Report

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16. Abstract

Forensic toxicology testing is an integral part of postmortem medicolegal investigations. In aviation casework, the results from these analyses are used to determine the impact, if any, of alcohols or drugs on the ability of a pilot to safely operate an aircraft. Quality Assurance/Quality Control (QA/QC) programs for forensic toxicology laboratories are necessary for maintaining confidence in analytical results and improving overall laboratory performance. Proficiency testing programs specifically targeting postmortem forensic laboratories are limited. In 1991, the Civil Aerospace Medical Institute, tasked with providing postmortem toxicology studies for the Federal Aviation Administration (FAA) and National Transportation Safety Board (NTSB), initiated a quarterly proficiency testing (PT) program specifically focused on the analysis of postmortem specimens like those obtained at autopsy following a fatal accident. Participants in this PT program used the results to gauge their laboratory's ability to correctly identify and quantify the target analytes. Two reviews of this program have been published, reporting on the findings for the first (July, 1991–April, 1998) and second (July, 1998–early April, 2005) seven-year periods. Similarly, this report is a compilation of the data from the final seven-year period (late April, 2005–December, 2012). A total of 30 PT challenge survey samples, including 14 urine samples, 9 bloods, and 7 liver tissue homogenates were submitted to participating laboratories during the seven-year period. The challenges either contained no analytes (negative) or were fortified with one or more alcohols/volatiles, drugs, or drug metabolites. The program, in some cases, add putrefactive amines and left samples at room temperature for 24 hours to initiate decomposition which is known to produce postmortem ethanol and putrefactive amines. An average of 26 laboratories participated in each of the surveys with an average of 22 (85%) submitting results back to CAMI. The data showed that 97.2% of laboratories submitting quantitative results were within 2 standard deviations of the overall mean value. When evaluated with a criterion of ± 20% of the overall analytical mean, the results were more varied but 85.4% of the labs met these criteria. There were some significant errors, including false positives for amphetamines, opiates, zolpidem, and metaxalone. Reporting these findings during a forensic investigation could have a major impact on the interpretation of toxicology results and the final disposition of a postmortem case. The program was discontinued after the December 2012 challenge.

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FAA POSTMORTEM FORENSIC TOXICOLOGY PROFICIENCY TESTING PROGRAM: THE FINAL SEVEN YEARS

INTRODUCTION

Postmortem medicolegal investigations are generally comprised of three primary areas of interest: investigation, autopsy, and laboratory sciences (e.g., pathology, microbiology, and toxicology). During the course of fatal aviation accident investigations in the United States, investigators from the National Transportation Safety Board (NTSB) and the Federal Aviation Administration (FAA) request that the local medical examiners/coroners submit postmortem fluid and tissue samples to the Civil Aerospace Medical Institute (CAMI; Oklahoma City, OK) for toxicology testing.[1-3] The samples are tested for drugs, volatiles, and combustion gases to determine their impact, if any, on the pilot's ability to safely operate the aircraft when the crash occurred. A correct determination of the cause of an aviation accident requires that all of the acquired data are as accurate as possible.

For forensic toxicology laboratories, including CAMI, good laboratory practice dictates that a quality assurance/quality control (QA/QC) program be in place to ensure accurate results.[4] Proficiency testing (PT) is an essential part of the QA/QC program and is required to maintain laboratory accreditation by organizations like the American Board of Forensic Toxicology.[5]

In July 1991, CAMI planned and executed a PT program for postmortem toxicology laboratories. In more recent years, it provided a second PT program to satisfy a requirement of the American Board of Forensic Toxicology (ABFT) for accredited laboratories to participate in 2 PT programs. Described in previous publications, this program was designed to challenge toxicology laboratories with postmortem specimens like those submitted by medical examiners, coroners, and aviation accident investigators. The program was voluntary with analytical results from each quarterly challenge being sent to the Biochemistry Research Team at CAMI for evaluation. The graded results were then sent back to participating laboratories to be used in their QA/QC programs for assessing their performance and making procedural corrections or improvements as necessary. The program was discontinued in 2012 as other PT programs were becoming available.

The findings from the first seven years (July 1991 to April 1998) and from the second seven-year period (July 1998 to early April 2005) have been published elsewhere.[6,7] This report summarizes the findings of the third and final seven-year period from late April 2005 to December 2012.

MATERIALS AND METHODS

Materials

Human urine, human blood, human liver and bovine liver were used for the sample preparation of the challenge samples for this segment of the proficiency test (PT) program. Certified drug-free human urine was purchased from UTAK Laboratories, Inc. (Valencia, CA). Human whole blood was purchased from the Oklahoma Blood Institute (Oklahoma City, OK). Human liver samples

were obtained from out dated cases at CAMI that were due to be destroyed. Bovine liver was purchased from the meat market of a local grocery store (Walmart, Oklahoma City, OK). Parent drug and their metabolites were purchased from Cerilliant Corporation (Austin, TX), Elsohly Laboratories, Inc. (Oxford, MS), Grace (formerly Alltech-Applied Science Labs; State College, PA), Sigma Chemical Company (St. Louis, MO), and United States Pharmacopeia (Rockville, MD).

PT Sample Preparation

A total of 30 samples were prepared over the 7-year PT program period (Table 1). Sodium fluoride, 1% by weight, was added to the human whole blood to prevent the putrefaction process from occurring. The certified drug-free human urine was not treated prior to PT sample preparation. When bovine liver was used for PT specimens, they were homogenized in DI water. The homogenate consisted of one part tissue to two parts water by weight.

Prior to use, each matrix type was screened by the CAMI Forensic Toxicology Research Team for ethanol/volatiles, prescription drugs, and drugs of abuse commonly used by humans. Bovine tissues were screened for drugs that are normally consumed by humans. Occasionally, veterinary medications normally administered to cattle may be detected in the bovine liver samples by routine testing procedures. Any positive findings during screening would result in the exclusion of that matrix type from the PT program or the positive finding would be disclosed to the participants as an "unintended analyte" and not graded.

The PT survey samples were prepared randomly in the human urine, blood, or bovine liver described previously. Selected analytes, potentially including both parent drugs and metabolites, were prepared in appropriate solvents in order to make a stock solution. The stock solution was added to urine, blood, or liver homogenate to simulate concentrations detected in typical cases examined by forensic toxicology laboratories (Table 1).[8-10] The samples were stored under refrigeration (4°C) for a minimum of 24 hours prior to shipment.

Decomposed (putrefied) samples are a common problem for postmortem toxicology laboratories presenting the analyst with potentially interfering substances. To simulate the putrefaction process, some samples were spiked with putrefactive bases, such as β-phenethylamine, tryptamine, and/or tyramine. Additionally, after drugs were added, selected survey samples were allowed to sit on a countertop at room temperature for at least 24 hours or longer prior to shipment in order to initiate putrefaction.

Sample Distribution and Result Summaries

PT samples were shipped in insulated cardboard boxes packed with frozen gel packs via Federal Express priority overnight shipment. Shipments occurred four times each year: January, April, July, and October. CAMI's Forensic Toxicology Research Team also participated in the PT study and its shipment was hand-delivered from the Biochemistry Research Team each quarter. The hand-receipt coincided with the date of delivery the other participants anticipated so as to disallow any extra time for analysis.

Table 1. PT survey data for the 2006–2012 survey period.

Sample number	Specimen type†	Analytes	Target Concentration	Mean (SD)	Range	Results within 2SD (%)	Results within 20% (%)	Qualitative only/ Quantitative	Participants	Respondents	Deferred
1	blood	Oxycodone	0.100 mcg/mL	0.102 (0.020)	0.050-0.130	91.7	83.3	3/12	28	25	1
		Methanol	7.9	10.0 (1.4)	9.0-12.0	**	**	0/3			
2	bovine liver	Acetaminophen	20.23 mcg/g	40.00 (10.40)	22.50-52.00	100	42.9	2/7	27	19	4
		Ethanol	236.7 mg/100g	17.57 (10.91)	200-250	100	100	2/3			
		Ibuprofen	44.32 mcg/g	219.67 (20.27)	10.0-33.0	**	0	0/9			
3	Urine	Sertraline	527 ng/mL	**	0.39 - 1.1	**	100	14/8	27	24	0
		Desmethylsertraline	1074 ng/mL	0.918 (0.147)	0.640 - 1.108	100	71.4	9/7			
		Ethanol	40 mg/dL	35.4 (4.1)	29.9 - 40.0	100	100	0/15			
4	Urine	MDA	50 ng/mL	55.5 (4.7)	49.0 - 60.0	**	**	2/4	27	24	1
		THC	10 ng/mL	6.9	**	**	**	0/1			
		THCCOOH	40 ng/mL	39.9 (11.8)	25.0 - 62.6	100	50	7/6			
		Methanol	36 mg/dL	38.5 (2.0)	35.0 - 42.0	100	100	0/13			
5	bovine liver	no drugs added ‡		53.7 (29.4)	20 - 96	**		0/13	27	22	7
6	Urine	Acetone	22 mg/dL	21 (3)	14 - 29	88.2	88.2	2/17	27	25	1
		Isopropanol	70 mg/dL	75 (12)	66 - 110	94.1	88.2	2/17			
		Ethanol	31 mg/dL	29 (5)	15 - 37	94.7	78.9	0/19			
7	blood	Butalbital	804 ng/mL	846(84)	700 - 960	100	100	9/9	27	25	1
		Diltiazem	120 ng/mL	**	60 - 190	**	40	2/5			
		Methadone	197 ng/mL	192(24)	150 - 233	100	85.7	8/13			
		Ethanol	79 mg/dL	75(4)	70 - 80	100	100	0/21			
8	blood	Fluoxetine	190 ng/mL	158(19)	127 - 180	100	100	6/9	27	25	1
		Norfluoxetine	200 ng/mL	112(12)	80 - 138	100	60	5/5			
		Methanol	31 mg/dL	33(4)	28 - 42	92.9	85.7	4/14			
9	bovine liver	no drugs added ‡		64.3	27 - 108			0/3	27	19	4
10	blood	Butalbital	600 ng/mL	610 (70)	500 - 720	100	100	10/9	27	23	1
		Chlorpheniramine	50 ng/mL	60(3)	50 - 60	**	**	10/4			
		Codeine	200 ng/mL	180(20)	110 - 200	92.3	92.3	6/13			
		Meprobamate	900 ng/mL	830(120)	700 - 1000	**	**	4/3			
		Ethanol	118 mg/dL	121(6)	110 - 138	95.5	100	0/22			
11	human liver	THC	50 ng/g	41	**	**	**	0/1	29	23	6
		THCCOOH	100 ng/g	96; 93	**	**	**	1/2			
		Ethanol	39 mg/hg	34(5)	27 - 40	100	100	2/7			
		Methanol	79 mg/hg	69(11)	57 - 92	91.7	83.3	1/12			
12	human liver	THC	100 ng/g	90		**	**	0/1	29	24	6
		THCCOOH	200 ng/g	126.5(44)	72 - 188	**	**	1/4			
13	urine	no drugs added							28	24	1
14	urine	d-amphetamine §	50 ng/mL	66(11) mcg/mL	46 - 89	100	75	15/8	28	24	6
		l-amphetamine	24 ng/mL								
		d-methamphetamine	74 ng/mL	73(12	47 - 85	87.5	87.5	15/8			
		phentermine	51 ng/mL	51(5)	44 - 56	**	**	15/3			

Sample number	Specimen type†	Analytes	Target Concentration	Mean (SD)	Range	Results within 2SD (%)	Results within 20% (%)	Qualitative only/ Quantitative	Participants	Respondents	Deferred
15	blood	carbamazepine	1500 ng/mL	1620 (260)	1300-2000	100	71.4	7/7	27	22	1
		Lorazepam	100 ng/mL	88 (19)	60-120	100	77.8	0/9			
		Triazolam	50 ng/mL	45 (4)	40-50	**	**	1/4			
		Ethanol	130 mg/dL	132 (8)	121-148	90.5	100	0/21			
16	urine	Fentanyl	60 ng/mL	63 (6)	58-72	**	**	12/4	27	24	1
		Phencyclidine	45 ng/mL	45 (4)	44-52	100	100	13/9			
		Methanol	8 mg/dL	9 (1)	8-10	**	**	1/3			
17	urine	Morphine	300 ng/mL	209(74)	84-294	**	**	11/10	27	26	1
		Oxycodone	100 ng/mL	91 (10)	75-107	100	100	9/8			
		Tramadol	200 ng/mL	202 (37)	139-274	100	77.8	13/9			
		Methanol	16 mg/dL	19 (2)	14-23	90.9	81.8	2/11			
18	urine	no drugs added							25	22	1
19	blood	no drugs added							25	21	1
20	blood	Cimetidine	500 ng/mL	**	**	**	**	2/0	24	20	1
		Phentermine	200 ng/mL	212 (16)	190-200	100	100	4/7			
		Phenytoin	10 mcg/mL	9.4 (1.4)	7.3-11.1	100	83.3	6/6			
		Ethanol	79 mg/dL	79 (5)	70-91	94.7	100	0/19			
21	blood	no drugs added							24	21	1
22	human liver	Carisoprodol	9.0 mcg/g	7.2 (1.1)	5.9-8.3	**	**	9/4	24	20	3
		Codeine	300 mcg/g	82 (64)	126-330	90	80	5/10			
		Dextrorphan	400 mcg/g	**	**	**	**	1/0			
		Ethanol	71 mg/hg	58 (7)	42-66	100	90	3/10			
23	urine	Acetone	40 ng/mL	38 (4)	30-44	100	100	2/18	24	22	2
		Ethanol	125 mg/dL	121 (14)	87-165	90	90	0/20			
		Isopropanol	50 mg/dL	47 (5)	37-56	100	100	2/18			
		Methanol	30 mg/dL	31 (2)	26-34	92.9	100	3/14			
24	human liver	Venlafaxine	400 ng/mL	504 (86)	420-652	100	80	6/5	24	19	5
		O- Desmethylvenlafaxine	150 ng/mL	145	**	**	**	0/1			
		N,O Didesmethylvanlafaxine	300 ng/mL	**	**	**	**	0/0			
25	blood	no drugs							23	21	2
26	urine	no drugs							23	20	0
27	urine	Amphetamine	200 ng/mL	208 (38)	150-270	100	60	2/5	23	19	1
		Phencyclidine	100 ng/mL	97 (8)	89-110	100	100	7/10			
28	urine	no drugs							23	19	1
29	urine	Acetaminophen	20 mcg/mL	21 (3)	17-24	100	100	3/4	23	20	3
		Phenytoin	10 mcg/mL	9 (1)	8--11	100	83.3	6/6			
		Methanol	158 mg/dL	143 (7)	139-166	100	100	1/15			
		Ethanol	79 mg/dL	78 (4)	70-87	94.1	100	0/17			
30	urine	no drugs							23	19	1

†All samples were human unless otherwise noted.

‡ Ethanol detected due to putrefaction.

** Data not available or too few data points for statistical analysis.

§ No labs distinguished *d* and *l* amphetamine.

Participating laboratories were instructed to return their analytical report sheets by a due date, even if no analysis was performed. This "no analysis" response would indicate whether all laboratories received their shipments as expected.

Analytical reports included an area to document analyte, quantitative or qualitative results, extraction procedure(s), and analytical method(s). A laboratory may also select to defer its analysis indicating one of two reasons. Either it "does not perform analysis on this specimen type" or "chooses not to perform analysis due to other reasons." On occasion, there were obvious clerical, transcriptional, or typographical errors such as misplaced decimal points or incorrect units. These errors were omitted from the quantitative statistics in this report but were included in the qualitative statistics.

CAMI PT Program personnel generated a summary report based on PT results within four weeks of the survey response deadline. These reports were mailed to all participating laboratories via the U.S. Postal Service.

RESULTS

Over the 7-year period of this report, there were an average of 26 participating laboratories for the quarterly challenges. This number is similar to the two previous surveys of the program.[6,7] As can be seen in the representative challenge in Figure 1, a variety of laboratories participated in the program during this final seven-year segment, including corporate and government facilities. The response rate for the challenges was good, with an overall mean of 22 (85.4%) of these laboratories submitting results back to the program for grading (Figure 2). The response rate held steady between 82 and 89% for the study period. The reason for a participant laboratory receiving PT samples but not responding was unknown in most cases. Reasons for not meeting the reporting deadline may include lack of analytical methodology for the required specimen type, instrument malfunction, difficulty with analyses, or insufficient personnel.

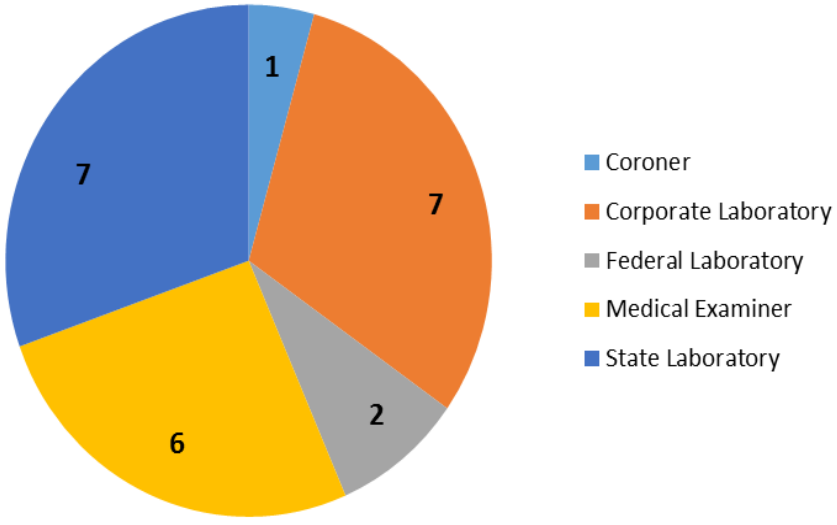

Figure 1. Demographic data from a representative quarterly challenge demonstrating the types of laboratories participating in the CAMI PT program.

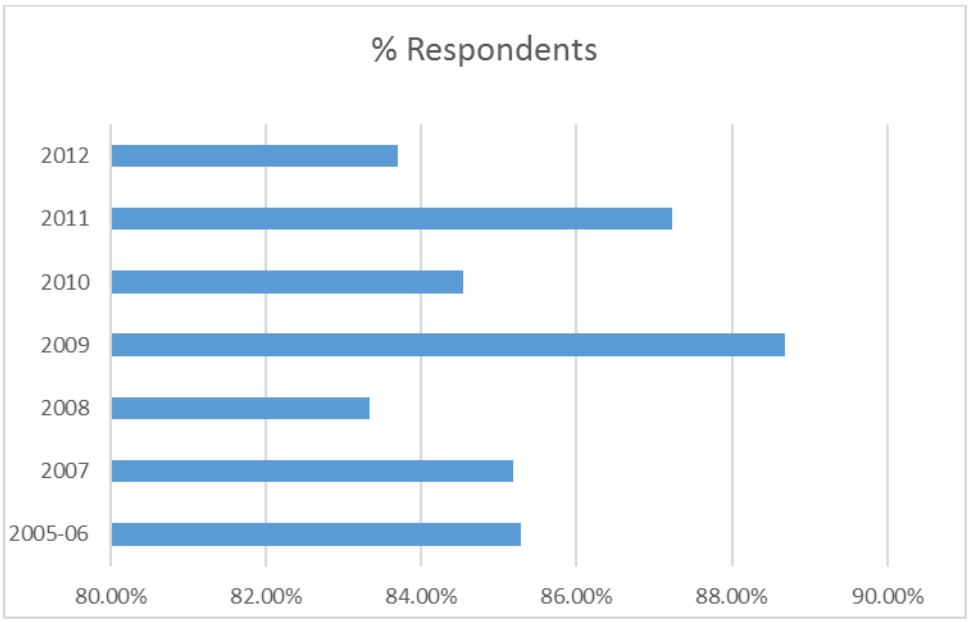

Figure 2. Percentage of respondents during the seven-year survey (mean, 85.4%; median, 85.2%). Two challenges from 2005 were included in this survey for completeness.

Evidence of putrefaction was detected in samples 5 and 9, both bovine liver samples. Sample 5 had been allowed to sit at room temperature for approximately 24 hours to initiate decomposition. The mean concentration of ethanol found by 13 laboratories was 53.7 (± 29.4) mg/dL with a range of 20–96 mg/dL. Sample 9 was not prepared to simulate decomposition, but 3 laboratories did report ethanol ranging from 27 to 108 mg/dL. These results are more than likely explained by the formation of ethanol during the decomposition initialized by the environmental conditions of shipping and storage.

When quantitative values were submitted, a review of Table 1 shows considerable agreement with the target concentrations. A mean of 97.2% of the laboratories, excluding those with obvious clerical errors and challenges with fewer than 5 results, returned quantitative values within 2 standard deviations (SD) from the overall analytical mean. Also, a mean of 85.4% of the participants reported values within ± 20% of the overall analytical mean, an accepted measure of accuracy in forensic toxicology. These findings are of particular interest for postmortem laboratories in that true postmortem samples are difficult to analyze for the reasons discussed in this report.

Interestingly, there were a number of notable false positives for drugs reported during the seven-year challenge period (Table 2). These false positive results illustrate the reason for confirmatory testing and the great care that a forensic toxicology laboratory must take to report accurate findings. A single laboratory in challenge sample number 2 reported amphetamines to be present in the bovine liver by fluorescence polarization immunoassay. In challenge sample 7, a laboratory, using gas chromatography/mass spectrometry, report 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), a metabolite of methadone, in the human blood. In sample 12, a human liver homogenate, there were multiple false positives (tramadol, opiates, doxylamine,

metaxalone, and acetone). Carbamazepine-11,10-epoxide was detected in blood sample 15. A laboratory reported gabapentin in sample 16, a human urine sample. Dextromethorphan and bupivacaine were reported in sample 17 and zolpidem in sample 21. Pramoxine and tapentadol were reported in samples 24 (human liver) and 25 (human blood).

Table 2. False positives detected on the PT program survey, 2006-2012.

Sample number	Specimen type*	Expected Analytes	False positives of note	Analytical Method
2	bovine liver	Acetaminophen Ibuprofen	Amphetamines	fluorescence polarization
7	blood	Butalbital Diltiazem Methadone Ethanol	EDDP	GC/MS
12	human liver	THC THCCOOH	Tramadol Opiates doxylamine metaxalone Acetone	GC, GC/MS
15	blood	Carbamazepine Lorazepam Triazolam Ethanol	Carbamazepine-10,11-epoxide	HPLC
16	urine	Fentanyl Phencyclidine Methanol	Gabapentin	LC/MS/MS
17	urine	Morphine Oxycodone Tramadol Methanol	Dextromethorphan Bupivacaine	GC/MS
21	blood	no drugs added	Zolpidem	LC/MS/MS
24	human liver	Venlafaxine O- Desmethylvenlafaxine N,O Didesmethylvanlafaxine	Pramoxine	GC/MS
25	blood	no drugs	Tapentadol	GC/MS

DISCUSSION

The PT program described here was started in 1991 and served the forensic toxicology community until 2012. This program was offered free of charge by the FAA's Civil Aerospace Medical Institute (CAMI) in Oklahoma City, Oklahoma. It was a well-received and respected effort to assist the laboratories with reaching the highest degree of quality and proficiency in their analytical work. As with the previous 14 years, a wide variety of laboratories with various functions in forensic and clinical sciences participated in this final segment. While there were other PT programs offered to the community from other sources, for many years this was the only program to provide samples of postmortem tissues. This report describes the work and results from that final segment of the program.

The PT program attempted to include analytes that a majority of postmortem forensic toxicology laboratories would see on a routine basis (Table 1). Drugs of abuse such as the primary psychoactive component of marijuana, delta-9-tetrahydrocannabinol (THC) and its major metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH) were included along

with methamphetamine, amphetamine, and phencyclidine (PCP). In addition, the program challenged the laboratories with samples containing prescription medications in therapeutic or sub-therapeutic concentrations. Medications with potential performance impairing effects are of particular interest to forensic toxicologists and were represented in this seven-year segment with analytes such as butalbital, lorazepam, methadone, phentermine and triazolam. Over-the-counter pharmaceuticals were represented by the drugs chlorpheniramine and cimetidine.

The mean response rate for the final 7-year segment was 85.4% (Figure 2). Reasons for a laboratory receiving samples and not submitting results were not provided by the non-participants. Changes in personnel, instrument malfunction, analytical method failure, and inability to meet the deadline due to laboratory workload are all possible explanations. Some laboratories did respond by submitting reports and marked "deferred" for a challenge. If a laboratory decided to defer its analysis, it would indicate one of two reasons. Either it "does not perform analysis on this specimen type" or "chooses not to perform analysis due to other reasons."

The number of laboratories submitting qualitative and quantitative responses varied considerably from one challenge to the next. For example, up to 20 laboratories submitted quantitative results for the analytes in the urine sample for challenge #23 (Table 1). The next challenge (#24) was a human liver sample containing venlafaxine and its metabolite, O-desmethylvenlafaxine. Only five laboratories quantitated the parent venlafaxine and only one performed a quantitative analysis for the metabolite. This could be due to a lack of an analytical method for these analytes in some laboratories. However, this also illustrates an issue common to forensic toxicology. The analysis of tissues is a difficult challenge for forensic laboratories as extraction and analysis of the drugs can be hindered by the complexity of the matrix. This can be compounded when decomposition has taken place, adding putrefactive interferences to the problem.

There were a number of unintended analytes reported over the seven-year period (Table 2). A cursory examination of the data would conclude that these unexpected findings are a cause for concern. When evaluated more closely, however, there may be a valid reason for at least some of the unintended findings. For example, the amphetamines found in bovine liver sample 2 may be explained by decomposition of the liver sample, as the method used was a non-specific immunoassay. The finding of EDDP in blood specimen 7 is likely explained by the result of the conversion of one of the expected analytes, methadone, to EDDP.[11] The presence of carbamazepine-10,11-epoxide in the human blood of sample 15 (Table 2) is difficult to explain. Human blood, urine, and liver were used to prepare the remaining challenge samples in Table 2. While they were initially screened for drugs and found to be negative, the drugs may have been present in concentrations below the limit of detection for the CAMI analytical methods but not for the participant procedures. This difference may be attributed to different missions of the various laboratories.

In order to achieve accreditation and withstand professional and judicial scrutiny, a forensic laboratory must have a comprehensive QA/QC program in place. The CAMI PT program served

as a tool for forensic toxicology laboratories to monitor the quality of their work product. The CAMI PT program specifically addressed postmortem laboratory practices and procedures by providing authentic postmortem samples. The program was recommended by the American Board of Forensic Toxicologists for laboratories to use to fulfill their accreditation requirement of PT program participation. From 1991 to 2012, the CAMI PT program was a respected national survey that served a variety of forensic organizations with different missions and improved the quality of forensic toxicology.

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