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ABSTRACT

The collection site and manner in which postmortem blood is collected and shipped to the reference laboratory in a forensic pathology autopsy setting may significantly impact toxicological results due to possible degradation of specimen or postmortem re-distribution (PMR). Generally, heart blood and peripheral blood specimens are collected; heart blood is often used for screening, while peripheral blood concentrations may help facilitate interpretation. Some offices perform an external "blind" stick of the inguinal region assuming the collection is from the femoral vein. Others rely on direct visual stick of the common iliac vein once the abdominal/pelvic cavity is opened. The assumption is that the method of acquisition does not influence drug concentration. Few studies have examined the influence of specimen collection and shipping procedures on postmortem drug concentration.

We examined three different peripheral blood specimen collection/shipping procedures in order to determine whether significant differences in drug concentration could be detected. Femoral blood (Specimen 1) was obtained by "blind" stick of the femoral vein in the area of the inguinal region and shipped to the laboratory at ambient temperature. A second blood specimen (Specimen 3) was obtained by a "blind" stick of the same side femoral vein in the inguinal region and was shipped to the reference laboratory on dry ice. Iliac blood (Specimen 2) was collected by direct visualization and clamping of the opposite side iliac vein (after opening the abdominal cavity) and shipped to the laboratory on dry ice. Specimens were analyzed by the reference laboratory by standard protocol. Drug concentrations were determined by gas chromatography/mass spectrometry (GCMS) or liquid chromatography/tandem mass spectrometry (LCMSMS). Data were analyzed using repeated measures analysis of variance

(ANOVA, $p < 0.05$). Collection/shipping procedure was the independent variable and drug concentration was the dependent variable.

Femoral, iliac and inguinal specimens (438 total) were collected from 146 decedents of which 14 cases had no detected drugs and 20 cases were not tested. The total number of cases used for analysis, therefore, was 112, in which 78 different illicit, prescription and over-the-counter drugs and/or their metabolites were detected. Multiple drugs and or metabolites were detected in most subjects, yielding 1-37 total drug detections of a single analyte. The most commonly detected analytes were alprazolam (*n=37*), 7-aminoclonazepam (*n=21*), morphine (*n=19*), oxycodone (*n=16*), ethanol (*n=15*), diphenhydramine (*n=14*), citalopram (*n=14*), delta-9-tetrahydrocannabinol (*n=14*) and methadone (*n=13*). Only delta-9-carboxy-THC (cannabis metabolite THCCOOH, $n = 12, p = 0.021$), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite, EDDP, $n = 9, p = 0.001$), and amphetamine ($n = 5, 0.021$) concentrations were significantly different between the three collection/shipping procedures ($p < 0.05$). Post Hoc analyses revealed that the inguinal specimen shipped on dry ice (Specimen 2) had significantly lower analyte concentrations than Specimen 1 or Specimen 3. Trends toward significance ($p < 0.10$) were determined for nortiptyline ($n = 10, p = 0.065$), N-desmethyltramadol ($n = 8, p = 0.074$), buprenorphine ($n = 3, p = 0.067$) and norbuprenorphine ($n = 2, p = 0.067$). For multiple subjects, one or more of the three specimens yielded negative results for one or more analytes. Concentration ratios comparing the three specimens were generally near 1.0; however, large variability was found. For multiple specimen ratios, concentrations were below 0.5 or higher than 2.0, indicating potential PMR.

While we detected no statistical difference between the three collection/shipping methods for most drugs, several subjects had one or two specimens that were negative. These findings

demonstrate potential for misinterpretation due to how the specimen was collected and or shipped to the reference laboratory and also suggest possible PMR within the peripheral compartment.

Table of Contents

EXECUTIVE SUMMARY

Introduction

Deaths investigated by the medical examiner and coroner offices across the United States impact public health, public safety and the criminal justice systems. Non-natural deaths, such as overt homicides or deaths suspicious in nature also are investigated by agents in the public safety realm. Autopsy information is used by both prosecutors and defense attorneys in civil and criminal trials.

Postmortem toxicology testing is a vital part of the forensic autopsy. Usually, the autopsy investigation includes investigation of the death scene noting the presence and types of prescription and illicit drugs, review of medical records, an autopsy examination, and testing of postmortem fluids and tissues for qualitative and quantitative analysis of drugs. Toxicology test results are interpreted in conjunction with autopsy findings and circumstances surrounding the death. Although a postmortem drug level appears to be concrete and easily interpreted, numerous variables prior to analyzing the specimen (pre-analytical) and during the analysis (analytical) can affect this number.

Acquisition of postmortem specimens used for toxicology testing is usually achieved by aspirating blood from the femoral vein and from the heart, collecting any urine from the bladder and collecting vitreous fluid. Tissues such as liver, kidney, brain and skeletal muscle may also be utilized for toxicology testing under certain conditions. Femoral venous blood is usually collected externally via a “blind stick” into the inguinal region, often with vigorous massaging of the leg to induce blood flow. These “blind sticks” introduce the possibility of acquiring a mixture of femoral venous and arterial blood. Alternatively, peripheral blood may be collected by direct

visualization and aspiration internally of the iliac vein, again often with vigorous massaging of the leg. Such massaging of the leg may dilute pure femoral venous blood with peripheral and, possibly, central venous blood. Careful collection of blood from a specific site in the body is important because some drugs may undergo significant redistribution within the body in the postmortem interval.

Another potential challenge in postmortem drug level interpretation involves the conditions in which postmortem blood samples are stored and shipped. Many coroner and medical examiner offices send specimens out of state to a reference laboratory. The specimens are transported to the reference laboratory by a courier service. At the Iowa Office of the State Medical Examiner, specimens are initially refrigerated at 4 degrees Celsius prior to transport and then shipped in ambient conditions. Specimens are refrigerated during the testing process (between1 and 8 weeks) and placed in long-term storage. Freezing of the specimens immediately after acquisition at autopsy would reduce the possibility of analyte degradation. Unfortunately, many coroner and medical examiner offices do not have the proper equipment or storage space to freeze specimens prior to transport. Also, shipping specimens on dry ice significantly increases the workload and cost of shipping.

There is an abundance of published literature discussing postmortem drug redistribution (PMR) and *in vitro* drug stability. Many of these studies concentrate on developing more sensitive and more efficient means of detecting certain drugs; however, there are substantially fewer studies examining the influence of pre-analytical conditions on the quality of a specimen prior to arrival to a reference laboratory. Studies examining PMR have usually concentrated on one particular drug such as various narcotics or other classes of drugs. To date, there have been

no large prospective studies specifically examining the possible differences in drug concentrations in multiple peripheral blood specimens.

Materials and Methods

Over a span of two years, we obtained blood from 146 decedents to evaluate the influence of specimen collection/shipping method on drug concentrations in three peripheral blood specimens. Of these, 112 were included in the study. The Iowa Office of the State Medical Examiner is charged with the investigation of non-natural manners of death including sudden, suspicious and unexplained deaths. Forensic autopsies are conducted in a state-of-the-art facility in Ankeny, Iowa. In 2009, the cause of death for approximately 11% of decedents autopsied at this facility was directly attributed to drug toxicities. Drugs of medicolegal significance such as narcotics, stimulants, and antidepressants not directly contributing to death were nonetheless present in a larger percentage of cases. Similarly, in the last full fiscal year of this study, 2012, the cause of death for approximately 13% of decedents autopsied at this facility was directly attributed to drug toxicities. All specimens collected in this study were submitted to the American Institute of Toxicology (AIT Laboratories) in Indianapolis, Indiana for testing. All subjects for this study had past medical history or findings at the scene suggested a drug-related death. Victims of homicide or suspected homicide were excluded. Decedents undergoing decomposition were also excluded. This study was considered exempt from review by an IRB committee; however, we felt it was just and reasonable to pursue informed consent from the legal next-of-kin or representative prior to inclusion into the study.

Routine autopsy specimens included heart blood, femoral blood collected by a “blind” inguinal stick or by direct visualization of the iliac vein with or without vigorous milking of the leg depending on ease of acquiring blood, urine and vitreous fluid. Of these routine specimens,

only the femoral blood specimen, acquired by "blind" stick of the inguinal region (Specimen 1) was evaluated in this study. Specimen 1 was placed in a gray-top Vacutainer® tube containing sodium fluoride preservative and refrigerated until shipped at ambient temperature in a standard cardboard box container with thin Styrofoam insulation. Samples were refrigerated up to two days prior to shipping and were shipped in ambient conditions via a commercial courier (FedEx). On arrival at AIT, the specimens were accessioned, refrigerated during testing and placed in long-term -20°C storage.

On the selected cases, two additional peripheral blood specimens (iliac and inguinal) were obtained. Iliac blood (Specimen 2) was obtained by opening the abdominal cavity, isolating the iliac vein on the opposite side from which the femoral vein specimen had been taken. Its proximal and distal segments were clamped and blood was collected by direct visualization without manipulation of the leg. The inguinal specimen (Specimen 3) was collected on the same side as the original femoral specimen (Specimen 1), without direct visualization or clamping of the vessel. The average volume of blood collected from the femoral and iliac sites (Specimens 2 and 3) was 5.7 mL (range, 2.0-10.0 mL). Specimens 2 and 3 were placed in gray-top Vacutainer® tubes with sodium fluoride preservative and were immediately placed in a -60°C freezer that consistently maintained temperature at -57°C. If the case was selected for the study, based on the toxicological examination of Specimen 1, Specimens 2 and 3 were shipped on dry ice to the reference laboratory.

Specimens were analyzed at AIT according to standard operating procedures. Classical cannabinoids, opiates, and oxycodone/metabolite were screened by an enzyme linked immunosorbent assay (ELISA – Immunalysis Direct ELISA) on a Dynex Technologies DSX System Analyzer. All other analytes were screened on a Waters Acquity ultra performance liquid

chromatograph coupled to a Waters LCT Premier XE time of flight mass spectrometer (UPLC/ToF). Acetone, ethanol, isopropanol, and methanol were screened on a Hewlett Packard 5890 gas chromatograph with flame ionization detector (GC-FID).

Presumptive positive screening results in femoral, iliac, and inguinal blood specimens were confirmed via alternate, more specific methodologies including a Waters Acquity ultra-performance liquid chromatograph coupled with either a Waters TQD tandem quadrupole mass spectrometer or a Waters Quattro Premier XE tandem quadrupole mass spectrometer (UPLC/MS/MS). Others were confirmed and quantified on a Waters Alliance 2695 high performance liquid chromatograph with either a Waters 2487 ultraviolet detector or a Waters 2475 fluorescence detector (HPLC). Butalbital was confirmed and quantified on an Agilent 6890 gas chromatograph coupled to an Agilent 5937 mass spectrometer (GC/MS). Ethanol was confirmed and quantified on a Hewlett Packard 5890 gas chromatography with flame ionization detector (GC-FID). Carbon monoxide was confirmed and quantified on an IL-682 CO-Oximeter. All analytical methods were validated according to laboratory standard operating procedures. Parameters assessed included limit of detection (LOD), limit of quantification (LOQ), upper limit of linearity (ULOL), linearity, imprecision and accuracy at the analytical cutoff, matrix selectivity, exogenous drug interferences, ion suppression (when necessary), and carryover. Analytical cutoffs were determined during validation testing and were relevant to postmortem toxicology.

Data were analyzed by repeated measures analysis of variance (ANOVA, $p < 0.05$) using EZAnalyze© 3.0, statistical analysis software for Microsoft® Excel. Collection/shipping procedure was the independent variable and drug concentration was the dependent variable.

Results

Femoral, iliac and inguinal specimens (438 total) were collected from 146 decedents during the period from January 2011 to February 2013. The cause of death was attributed to a single drug in 17 cases (12%), a mixture of multiple drugs in 51 cases (35%) inhalation of carbon monoxide in 2 cases (1%), inhalation of paint thinner in 1 case (1%) and inhalation of chloroform in 1 case (1%). Drugs were considered to be a significant other condition that contributed to death in 13 cases (9%). The manners of death were certified as follows: accidental (42%), natural (24%), suicide (23%) and undetermined (11%). The age range was 13 to 80 years old (mean age 42 years old). There were 91 males (62%) and 54 females (37%). Caucasians represented 96% of the subjects and African Americans represented 6%. The weight range was from 104 to 300 pounds (mean 195 pounds). The mean postmortem interval, defined as time last seen alive to time of specimen collection, was 39 hours.

Of the 146 cases in which specimens had been collected, 112 were included in the study. There were 34 cases that were collected but not included in the analysis. Of the 34 cases that were excluded from the analysis, in 6 cases the next-of-kin refused to give consent, in 14 cases the initial screening (Specimen 1) was negative for drugs, in 1 case the initial screening detected only chloroform, and in 13 cases toxicology testing of the study samples (Specimens 2 and 3) was not able to be completed due to running out of time before reaching the end of the project period. In the 112 cases, there were 62 different illicit, prescription and over-the-counter drugs and/or metabolites detected (Table 1). A single specimen triplet is defined as femoral, iliac and inguinal specimens, collected from a single decedent, that were analyzed for a single drug. Multiple drugs and or metabolites were detected in most subjects. Sixteen drugs/metabolites

were detected in a single specimen triplet each (Table 2). For the remaining drugs/metabolites, there were 2-37 specimen triplets analyzed.

Only delta-9-carboxy-THC (cannabis metabolite THCCOOH, $n = 12$, $p = 0.021$), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite, EDDP, $n = 9$, $p = 0.001$), and amphetamine ($n = 5$, 0.021) concentrations were significantly different between the three collection/shipping procedures ($p < 0.05$). Post Hoc analyses revealed that the inguinal specimen shipped on dry ice (Specimen 3) had significantly lower analyte concentrations than Specimen 1 or Specimen 2 for THCCOOH, EDDP and amphetamine. For amphetamine, inguinal blood was significantly lower than iliac blood. Trends toward significance ($p < 0.10$) were determined for nortiptyline ($n = 10$, $p = 0.065$), N-desmethyltramadol ($n = 8$, $p = 0.074$), buprenorphine ($n = 3$, $p = 0.067$) and norbuprenorphine ($n = 2$, $p = 0.067$).

There was large variability in drug concentrations within and between subjects. In 49 specimen triplets, the femoral blood specimen (Specimen 1) was positive while the iliac (Specimen 2), inguinal (Specimen 3) or both were negative (Table 3). This occurred for THC ($n = 9$), fluoxetine ($n = 4$), norsertraline ($n = 4$) hydromorphone ($n = 3$), norfluoxetine ($n = 2$), nortriptyline ($n = 2$), oxycodone ($n = 2$), promethazine ($n = 2$), sertraline ($n = 2$), and on a single specimen triplet for multiple other analytes (Table 3).

Within subject variability was characterized by calculating concentration ratios. We found large variability in iliac/femoral, inguinal/femoral and iliac/inguinal ratios, although concentrations between the three sites within a specimen triplet were generally very similar (Table 4). The mean ratios among all drug/metabolites were 1.0, 0.9 and 1.2 for iliac/femoral, inguinal/femoral and iliac/inguinal comparisons, respectively. The lowest ratio was 0.0 (one

specimen was negative), found in multiple specimen triplets (see Table 3) and the highest ratio was 10.9 found when comparing inguinal/femoral blood positive for THC.

Conclusions

Reliable interpretation of postmortem blood drug concentrations depends greatly on pre-analytical variables such as collection site and possible analyte instability. Blood collected from any peripheral site, regardless of collection method, is often considered to most closely approximate the ante-mortem drug concentration and is therefore a valuable specimen for interpretation. We found statistical difference between the three collection/shipping methods only for three analytes. Meaningful conclusions based on these findings remain suspect. While statistically significant differences were detected, small sample size should be considered. Overall, drug/metabolite concentrations were very similar between all three specimens. Nevertheless, methadone metabolite (2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine, EDDP), delta-9-carboxy-THC and amphetamine concentrations in the inguinal specimen were significantly lower than in the other two sites. One possible explanation may include analyte instability in this matrix after one freeze/thaw cycle. The inguinal specimen was collected from approximately the same site and immediately following the femoral blood specimen, but was frozen prior to testing. The two specimens differed only slightly in how they were collected. While taken from the same general location in the inguinal region, each was obtained by a different blind stick of the femoral vein. The femoral blood (Specimen 1) was temporarily refrigerated prior to submission to the laboratory and was shipped at ambient temperature shortly after autopsy. The inguinal specimen (Specimen 3), was immediately frozen at -60°C (consistently maintained at -57°C). Several weeks passed in some cases before informed consent

was obtained from next-of-kin. Laboratory results on the femoral specimen were received within approximately 1 week.

Delta-9-carboxy-THC concentrations may increase when stored at various conditions after collection. This may be explained by conversion of delta-9-carboxy-THC-glucuronide to delta-9-carboxy-THC at higher temperature. Therefore, our data may reflect an increase in delta-9-caboxy-THC when collected in the femoral vein and shipped at ambient temperature, instead of its decrease in the other two specimens maintained frozen.

Significantly lower amphetamine concentration in the inguinal specimen may be explained by site of collection and/or PMR. The short period in which the femoral blood was shipped at ambient temperature should not cause significant amphetamine concentration decreases. Post Hoc analysis revealed that the inguinal blood amphetamine concentration was significantly lower than the iliac blood concentration. Both of these specimens were collected and shipped to the laboratory after frozen storage. Given its relative stability, this finding suggests possible PMR. The iliac vein found within the abdominal cavity and some drugs may re-distribute into the iliac blood to a greater extent than more distal sites such as femoral blood.

Trends toward significance ($p < 0.10$) were determined for nortiptyline ($n = 10$, $p = 0.065$), N-desmethyltramadol ($n = 8$, $p = 0.074$), buprenorphine ($n = 3$, $p = 0.067$) and norbuprenorphine ($n = 2$, $p = 0.067$). As with the aforementioned analytes, different drug concentrations for nortriptyline, N-desmtehyltramadol, buprenorphine and norbuprenorphine, may be explained by analyte instability or potential PMR within the peripheral compartment.

There were multiple specimen triplets in which iliac or inguinal or both blood specimens were negative. Cases were selected based in part on whether femoral blood (Specimen 1) was positive for one or more drugs/metabolites. Drug concentrations in the remaining two specimens

were assumed to be positive, at least at the time of autopsy. Negative findings in these specimens suggests potential degradation of analytes in the frozen specimens (Specimens 2 and 3), or artificially elevated concentration in the initial specimen, owing to PMR. We examined concentration ratios between the three specimens to demonstrate within subject variability in analyte concentration (Table 4). Generally, concentrations were similar between the three specimens; however, some ratios were less than 0.5 or greater than 2.0. This indicates that preanalytical factors influence drug concentration and potentially toxicological interpretation. Therefore, while generally it appears that any of these collection/shipping procedures should yield similar results, there still is the potential for misinterpretation based on peripheral blood concentration or possibly the absence of drug detected. Usually, multiple specimens are collected during autopsy for toxicological determination. Heart blood, peripheral blood, urine, vitreous fluid, liver and other organ tissues may be collected. Drug/metabolite concentrations in peripheral blood remain an essential part of the overall toxicological investigation; however, concentrations in other specimen types also aid interpretation. These data further support the practice of basing interpretation on the totality of evidence, including examination of peripheral blood and other specimen types, as well as other investigative factors.

To our knowledge, there are no other prospective studies that have compared drug/metabolite concentrations in multiple peripheral blood sites. The total scope of this study, number of different drugs/metabolites detected and total cases has not been reported elsewhere. These data will be valuable for toxicologists, pathologists and clinicians in interpreting drug/metabolite concentrations amid other case findings.

The results and conclusions from this study have implications for forensic pathologists and toxicologists in approaching determination of death in possible drug-related fatalities.

Occasional widely varying drug concentrations and false negative results for specific drugs in compared specimens highlight the need to be cautious when interpreting postmortem drug levels. Such interpretation is best done as a team effort with input from pathologists and toxicologists and consideration of the totality of the case including circumstances of death, decedent's drug use and medical history, and scene investigation. The results from this study can potentially help guide the creation of standard operation procedures in terms of types and sites of specimen collection during routine autopsies.

1. INTRODUCTION

Deaths investigated by the medical examiner and coroner offices across the United States impact public health, public safety and the criminal justice systems. Statistics regarding the number of drug-related deaths across the United States are derived mainly from death certificates of which a significant number are derived from autopsy findings. Many deaths evaluated by the medical examiner or coroner are also investigated by agents in the public safety realm because the deaths are non-natural, such as overt homicides, or suspicious or sudden in nature. Autopsy information is used by both prosecutors and defense attorneys in civil and criminal trials.

Postmortem toxicology testing is a vital part of the forensic autopsy. Intentional and unintentional drug-related deaths are a significant percentage of any coroner or medical examiner's workload. A recent study by the Centers for Disease Control and Prevention found that poisoning, including prescription and illicit drugs, was the second leading cause of injury death (CDC 2007). The evaluation of suspected drug-related deaths falls under the jurisdiction of the medical examiner or coroner system. Usually, investigation of these deaths include investigation of the death scene noting the presence and types of prescription and illicit drugs, review of medical records, an autopsy examination, and testing of postmortem fluids and tissues for qualitative and quantitative analysis of drugs. Less often, an external examination of the body with collection of postmortem blood for toxicology testing instead of a complete autopsy is performed. Toxicology test results are interpreted in conjunction with autopsy findings and circumstances surrounding the death. The information derived from these examinations is utilized by the public safety, criminal justice and public health systems (Council 2009). Drug poisoning deaths involving prescription medication can lead to litigation against the prescribing physician. Drug poisoning deaths involving illicit drugs can lead to criminal charges against the

supplier of the drug. These charges are more likely to result in conviction if the evidence obtained from the autopsy, including postmortem toxicology results, is concrete and not subject to widely varying interpretation. Although a postmortem drug level appears to be concrete and easily interpreted, numerous variables prior to analyzing the specimen (pre-analytical) and during the analysis (analytical) can affect this number.

Acquisition of postmortem specimens used for toxicology testing is usually achieved by aspirating blood from the femoral vein, aspirating blood from the heart, collecting any urine from the bladder and collecting vitreous fluid. Tissues such as liver, kidney, brain and skeletal muscle may also be utilized for toxicology testing under certain conditions. Femoral venous blood is usually collected externally via a “blind stick” into the inguinal region, often with vigorous massaging of the leg to induce blood flow. These “blind sticks” introduce the possibility of acquiring a mixture of femoral venous and arterial blood. Another method of collection is by direct visualization and aspiration internally of the iliac vein, again often with vigorous massaging of the leg. Such massaging of the leg increases the potential of diluting pure femoral venous blood with peripheral and, possibly, central venous blood. Isolating and clamping the femoral vein to ensure that only femoral venous blood was collected would entail incising into the tissue of the thigh to locate the vessel, which is tedious and unnecessarily disfiguring. Careful collection of blood from a specific site in the body is important because some drugs may have significant redistribution within the body in the postmortem interval. Levels of certain drugs can differ depending on the site of acquisition of blood, i.e., femoral venous blood versus heart blood versus peripheral blood (Jones and Pounder 1987; Pounder and Jones 1990; Hilberg, Rogde et al. 1999; Pelissier-Alicot, Gaulier et al. 2003; Flanagan, Connally et al. 2005; Yarema and Becker 2005).

Another potential obstacle in postmortem drug level interpretation involves the conditions in which postmortem blood samples are stored and shipped. Many coroner and medical examiner offices do not have on-site toxicology labs to process specimens. Fluid and tissue samples are often sent out of state to a reference laboratory. The specimens are transported to the reference laboratory by a courier service. The conditions in which the specimens are transported can vary. At the Iowa Office of the State Medical Examiner, specimens are initially refrigerated at 4 degrees Celsius prior to transport and then shipped in ambient conditions. Once at the reference lab, the specimens are immediately accessioned, refrigerated during the testing process (up to 2 weeks) and placed in long-term storage. Ideally, freezing of the postmortem specimens after acquisition at autopsy would reduce any possibility of postmortem degradation of the specimens, which may negatively affect the stability of any drugs within the specimen. The specimens would need to be shipped on dry ice to maintain a frozen state, and thawed only prior to analysis. Unfortunately, many coroner and medical examiner offices do not have the proper equipment or storage space to freeze specimens prior to transport. Also, shipping specimens on dry ice significantly increases the workload and cost of shipping. Appreciable loss of certain drugs may occur during the postmortem interval prior to acquisition of the specimens at autopsy. It is known that certain drugs like cocaine and nitrobenzodiazepines are fairly unstable in the postmortem period and can degrade over time (Robertson and Drummer 1998; Drummer 2004; Skopp 2004). Such instability of drugs may be affected by shipping conditions of the specimens to a reference toxicology laboratory especially if the specimens undergo various temperature changes.

There is an abundance of published literature discussing postmortem drug redistribution (PMR) and *in vitro* drug stability. Many of these studies concentrate on developing more

sensitive and more efficient means of detecting certain drugs (Koves and Wells 1992; Logan, Friel et al. 1995; Gergov, Nokua et al. 2009; Jagerdeo, Schaff et al. 2009; Nielsen 2009; Taylo and Elliot 2009). There are substantially fewer studies examining the influence of pre-analytical conditions on the quality of a specimen prior to arrival to a reference laboratory (Skopp 2004; Linnet, Johansen et al. 2008). The physical separation of many reference toxicology labs from medical examiner or coroner offices and the budget restraints and workload experienced by most medical examiner and coroner offices may be largely to blame for this lack of investigation.

Review of the medical and toxicology literature reveals numerous studies examining PMR. These studies have usually concentrated on one particular drug such as various narcotics or other classes of drugs from deaths due to intentional drug intoxication with analysis being performed on the drug levels from blood obtained from various specific sites in the body (Kunsman, Rodriguez et al. 1999; Johnson, Lewis et al. 2007; Luckenbill, Thompson et al. 2008; Vance and McIntyre 2009). To our knowledge, only one study compared toxicology test results using a limited toxicology panel from blood obtained from clamped versus unclamped femoral vessels (Hargrove and McCutcheon 2008). This particular study used a limited toxicology panel looking at only 8 drugs from 4 different drug classes. The study also did not evaluate the possible influence on peripheral venous blood or possible contamination of femoral arterial blood. To date, there have been no large prospective studies specifically looking at the possible differences in various postmortem drug levels due to commonly performed blood sampling by external acquisition of femoral blood in association with vigorous massaging of the leg compared to careful acquisition of iliac venous blood internally after isolating the iliac vein prior to sampling.

Review of the medical and toxicology literature also reveals occasional references to pre-analytical degradation of certain drugs, namely cocaine and nitrobenzodiazepines (Moriya and

Hashimoto 1996; Robertson and Drummer 1998). Again, the literature largely consists of studies examining few drugs stored at various temperatures and analyzed after different times (Kunsman, Presses et al. 2000). To date, there have been no large prospective studies examining possible changes in postmortem drug levels due to differences in storage and shipping methods after acquisition.

This study design focused on two discrete pre-analytical conditions that might affect drug stability and concentration in postmortem specimens. Firstly, this study evaluated the degree that storage and shipping of blood specimens overnight via commercial carrier in ambient conditions affected the stability of any drugs when compared with careful preservation of the specimen by immediate freezing of the blood and shipping on dry ice to maintain the frozen state prior to analysis. Secondly, this study evaluated any changes in drug levels occurring with standard external acquisition of femoral blood ("blind" stick of the inguinal region), sometimes with vigorous massaging of the leg, a technique that is commonly used during many forensic external and internal autopsy examinations, as opposed to direct visualization and careful acquisition of blood from a single site, the iliac vein. Significant differences in either site-specific acquisition of blood or the method of preservation and transportation of blood specimens to a reference laboratory could impact the standard operating procedures of coroner and medical examiner offices concerning acquisition of toxicology specimens and storage and transport of specimens. The information derived from this study may impact interpretation of postmortem drug levels in the determination of cause of death and provide information for expert witnesses testifying in court on drug-related issues.

2. MATERIALS AND METHODS

2.1 Facilities

Over a span of two years, the Iowa Office of the State Medical Examiner, which provides autopsy coverage for approximately 95 counties in the state of Iowa, obtained blood from 146 decedents to evaluate the influence of specimen collection method and post-autopsy drug instability. The Iowa Office of the State Medical Examiner is charged with the investigation of non-natural manners of death including sudden, suspicious and unexplained deaths. Forensic autopsies are conducted in a state-of-the-art facility in Ankeny, Iowa. In 2009, the cause of death for approximately 11% of decedents autopsied at this facility was directly attributed to drug toxicities. Drugs of medicolegal significance such as narcotics, stimulants, and antidepressants not directly contributing to death were nonetheless present in a larger percentage of cases. Similarly, in the last full fiscal year of this study, 2012, the cause of death for approximately 13% of decedents autopsied at this facility was directly attributed to drug toxicities.

All specimens collected in this study were submitted to the American Institute of Toxicology (AIT Laboratories) in Indianapolis, Indiana for testing. AIT is a nationally recognized toxicology laboratory specializing in pain management, forensic, clinical, and pharmaceutical toxicology testing and research. The laboratory is accredited by the College of American Pathologists (CAP), Clinical Laboratory Improvement Amendments (CLIA), International Organization of Standardization (ISO) and the American Board of Forensic Toxicology (ABFT).

2.2 Case Selection

Selection of cases was dependent on the circumstances of death. The forensic pathologist concentrated on those decedents whose past medical history or findings at the scene suggested a drug-related death or who were likely to have prescription medications or illicit drugs in their blood at the time of death. Decedents who were victims of homicide or suspected homicide were excluded from the study. If postmortem acquisition of blood was insufficient for both routine toxicology testing and testing as part of the research project because of trauma or other causes of hypovolemia, these decedents were excluded from the study. Decedents undergoing decomposition were also excluded.

2.3 Informed Consent

Decedents are not considered "human subjects" for research purposes. Postmortem blood is normally obtained from various sites as part of a routine forensic autopsy, so no fluids or tissues not ordinarily retained occurred. In addition, toxicology tests are normally performed on postmortem blood or tissues as part of the standard forensic autopsy by the Iowa Office of the State Medical Examiner. Based on these conditions, this study was considered exempt from review by an IRB committee. Iowa code permits the retention of significant portions of tissues for diagnostic, research and teaching purposes without notification of next-of-kin (Iowa Code § 691.6(8)(2005)). Despite the generous allowances of the code to conduct research without the need for informed consent, the researchers felt it was just and reasonable to pursue informed consent from the legal next-of-kin or representative prior to inclusion of a particular decedent's specimens into this study. Informed consent was generally obtained by the research assistant whose salary was funded by this grant via telephone contact with the identified legal next-of-kin or representative.

2.4 Specimen Collection

Routine autopsy specimens included heart blood collected by visually directed stick of the inferior vena cava, femoral blood collected by a "blind" inguinal stick or by direct visualization of the iliac vein with or without massaging of the leg depending on ease of acquiring blood, urine and vitreous fluid. All blood specimens were collected prior to evisceration. Of these routine specimens, only the femoral blood specimen acquired by "blind" stick of the inguinal region (Specimen 1) was evaluated in this study. This specimen was placed in a gray-top Vacutainer® tube containing sodium fluoride and sent to AIT for testing as part of the routine casework. These samples were refrigerated but not frozen until they were shipped in standard cardboard box containers with thin Styrofoam insulation provided by AIT. Samples were held in refrigeration prior to shipping no more than 2 days after acquisition. The specimens were shipped in ambient conditions via a commercial courier (FedEx) by plane from Des Moines, IA, which is 20 minutes from the IOSME in Ankeny, IA, through the main FedEx hub in Memphis, TN to Indianapolis, IN prior to ground delivery to AIT. According to a service representative for FedEx, ambient temperatures on the planes vary according to type of plane, package location on the plane, and the cruising altitude. In general, most temperatures in flight are between 65 and 90°F; however, lower cargo and bulk on certain planes can reduce the temperature to 0 degrees Fahrenheit. On arrival at AIT, the specimens were accessioned, refrigerated during testing and placed in long-term -20°Cstorage.

On the selected cases, two additional peripheral blood specimens were obtained. Both specimens were collected and immediately placed in a gray-top Vacutainer® tube, containing sodium fluoride. Iliac blood (Specimen 2) was obtained by opening the abdominal cavity, isolating the iliac vein on the opposite side from which the femoral vein specimen had been

taken by clamping its proximal and distal segments, and aspirating any blood by direct visualization prior to any external aspiration of blood or manipulation of the legs. This specimen was immediately frozen by placement in a -60°C freezer (maintained consistently at -57°C) prior to transport to the reference laboratory. This specimen was shipped on dry ice to maintain its frozen state. After the blood from the iliac vein had been obtained, an external "blind stick" of the inguinal region from the same side as the femoral venous sample (Specimen 1) with or without massaging of the right leg and aspiration of presumed femoral venous blood was performed (Specimen 3). This specimen was shipped on dry ice to maintain its frozen state to the reference laboratory. Specimens 2 and 3 were maintained at -57°C until shipment to AIT Laboratories until results from the routine autopsy specimens had been received and informed consent had been obtained. If the case was selected for inclusion into the study, Specimens 2 and 3 were submitted to AIT Laboratories for testing. On average, 5.7 mL (range 2.0 – 10.0 mL) of blood was collected for Specimens 2 and 3.

Cases were given a second identification number in addition to the standard case identification label. The cases were labeled sequentially as they were entered into the study. The first case was labeled, "R-001," the second case was labeled, "R-002," the third case was labeled, "R-003," and so forth. In addition, the blood obtained from the clamped iliac vein was given the suffix of "-A." The research blood from the right leg that was frozen was given the suffix of "-B." For example, blood obtained from the clamped iliac vein from the first case was labeled, "R-001-A."

2.5 Testing

Specimens were analyzed at AIT according to standard operating procedures. Classical cannabinoids, opiates, and oxycodone/metabolite were screened by an enzyme linked

immunosorbent assay (ELISA – Immunalysis Direct ELISA) on a Dynex Technologies DSX System Analyzer. Amphetamines, analgesics, anesthetics, antibiotics, anticonvulsants, antidepressants, antipsychotics, barbiturates, benzodiazepines, cardiovascular agents, cocaine/metabolites, endocrine agents, fentanyl, gastroenterology agents, hypnotics, methadone/metabolite, narcotics, neurology agents, phencyclidine, propoxyphene/metabolite, sedatives, stimulants, and urology agents were screened on a Waters Acquity ultra performance liquid chromatograph coupled to a Waters LCT Premier XE time of flight mass spectrometer (UPLC/ToF). Acetone, ethanol, isopropanol, and methanol were screened on a Hewlett Packard 5890 gas chromatograph with flame ionization detector (GC-FID).

Presumptive positive screening results in femoral, iliac, and inguinal blood specimens were confirmed via alternate, more specific methodologies. 6-acetylmorphine, 7-aminoclonazepam, alpha-PVP, alprazolam, amitriptyline, amlodipine, amphetamine, benzoylecgonine, buprenorphine, bupropion, carisoprodol, chlordiazepoxide, clomipramine, clonazepam, clozapine, codeine, cyclobenzaprine, demoxepam, dextromethorphan, diazepam, diphenhydramine, doxepin, doxylamine, duloxetine, EDDP, fentanyl, fluoxetine, gabapentin, hydrocodone, hydromorphone, hydroxyzine, levetiracetam, lidocaine, lorazepam, meprobamate, methadone, methamphetamine, methylphenidate, metoprolol, midazolam, mirtazapine, morphine, norbuprenorphine, norclomipramine, norclozapine, nordiazepam, nordoxepin, norfluoxetine, norsertraline, nortramadol, nortriptyline, norvenlafaxine, olanzapine, oxazepam, oxycodone, oxymorphone, paroxetine, pregabalin, pseudoephedrine, quetiapine, rocuronium, sertraline, temazepam, THC, THC-COOH, tramadol, valproic acid, venlafaxine, warfarin, and zolpidem were confirmed and quantified on a Waters Acquity ultra-performance liquid chromatograph coupled with either a Waters TQD tandem quadrupole mass spectrometer or a

Waters Quattro Premier XE tandem quadrupole mass spectrometer (UPLC/MS/MS). Acetaminophen, carbamazepine, carbamazepine-10,11-epoxide, citalopram, lamotrigine, promethazine, and trazodone were confirmed and quantified on a Waters Alliance 2695 high performance liquid chromatograph with either a Waters 2487 ultraviolet detector or a Waters 2475 fluorescence detector (HPLC). Butalbital was confirmed and quantified on an Agilent 6890 gas chromatograph coupled to an Agilent 5937 mass spectrometer (GC/MS). Ethanol was confirmed and quantified on a Hewlett Packard 5890 gas chromatography with flame ionization detector (GC-FID). Carbon monoxide was confirmed and quantified on an IL-682 CO-Oximeter (CO-OX).

All analytical methods utilized were validated according to laboratory standard operating procedures. Parameters assessed during method validation for qualitative screening assays included limit of detection (LOD), imprecision and accuracy at the analytical cutoff, matrix selectivity, exogenous drug interferences, and carryover. Parameters assessed during method validation for quantitative confirmatory assays included linearity, limit of detection (LOD), lower limit of quantitation (LLOQ), and upper limit of quantitation (ULOQ), imprecision and accuracy, matrix selectivity, exogenous drug interferences, ion suppression (when necessary), and carryover. Analytical cutoffs were determined during validation testing and were relevant to postmortem toxicology.

2.6 Statistical Methods

Data were analyzed by repeated measures analysis of variance (ANOVA, $p < 0.05$) using EZAnalyze© 3.0, statistical analysis software for Microsoft® Excel. Collection/shipping procedure was the independent variable and drug concentration was the dependent variable.

3. RESULTS

3.1 Demographics

Femoral, iliac and inguinal specimens (438 total) were collected from 146 decedents during the period from January 2011 to February 2013. The cause of death was attributed to a single drug in 17 cases (12%), a mixture of multiple drugs in 51 cases (35%) inhalation of carbon monoxide in 2 cases (1%), inhalation of paint thinner in 1 case (1%) and inhalation of chloroform in 1 case (1%). Drugs were considered to be a significant other condition that contributed to death in 13 cases (9%). The manners of death were certified as follows: accidental (42%), natural (24%), suicide (23%) and undetermined (11%). The age range was 13 to 80 years old (mean age 42 years old). There were 91 males (62%) and 54 females (37%). Caucasians represented 96% of the subjects and African Americans represented 6%. The weight range was from 104 to 300 pounds (mean 195 pounds). The average postmortem interval, defined as time last seen alive to time of specimen collection, was 39 hours. Obtaining the initial femoral blood specimen by vigorous massaging was required in only 11 cases.

3.2 Drugs Detected

Of the 146 cases in which specimens had been collected, there were 112 cases in which 62 different illicit, prescription and over-the-counter drugs and/or their metabolites were detected (Table 1). A single specimen triplet is defined as femoral, iliac and inguinal specimens, collected from a single decedent, that were analyzed for a single drug. Multiple drugs and or metabolites were detected in most subjects. Sixteen drugs/metabolites were detected in a single specimen triplet each (Table 2). For the remaining drugs/metabolites, there were 2-37 specimen triplets analyzed.

3.3 Statistical Findings

Only delta-9-carboxy-THC (cannabis metabolite THCCOOH, *n = 12, p = 0.021*), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite, EDDP, *n = 9, p = 0.001*), and amphetamine (*n = 5, 0.021*) concentrations were significantly different between the three collection/shipping procedures ($p < 0.05$). Post Hoc analyses revealed that the inguinal specimen shipped on dry ice (Specimen 3) had significantly lower analyte concentrations than Specimen 1 or Specimen 2 for THCCOOH, EDDP and amphetamine. For amphetamine, inguinal blood was significantly lower than iliac blood. Trends toward significance ($p < 0.10$) were determined for nortiptyline (*n = 10, p = 0.065*), N-desmethyltramadol (*n = 8, p = 0.074*), buprenorphine (*n = 3, p = 0.067*) and norbuprenorphine (*n = 2, p = 0.067*).

Table 1: Summary of drugs and/or metabolite concentrations (ng/mL) in 112 decedents (457 specimen triplets). Femoral blood was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood was collected by direct visualization and clamping the vein. Inguinal blood was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Alprazolam	37	Femoral	54.9 ± 68.7	37.7	2.5 - 364.0	.536
		Iliac	52.4 ± 54.1	36.7	2.6 - 229.0	
		Inguinal	50.7 ± 51.5	35.9	3.5 - 228.0	
7-aminoclonazepam	21	Femoral	41.6 ± 31.6	33.5	42.9 - 114.0	.536
		Iliac	47.4 ± 34.6	48.2	21.0 - 162.0	
		Inguinal	50.5 ± 36.3	38.0	18.5 -169.0	
Morphine	19	Femoral	282.9 ± 232.5	226.0	31.5 - 863.0	.424
		Iliac	345.1 ± 339.8	239.0	24.0 - 1413.0	
		Inguinal	279.2 ± 243.4	203.0	27.6 - 953.0	
Oxycodone	16	Femoral	227.3 ± 300.7	100.0	10.1 - 1137.0	.565
		Iliac	220.3 ± 261.6	100.4	11.3 - 934.0	
		Inguinal	195.7 ± 200.7	103.3	0.0 - 526.0	
Ethanol (%w/v)	15	Femoral	0.15 ± 0.13	0.09	0.03 - 0.43	.890
		Iliac	0.15 ± 0.13	0.10	0.03 - 0.47	
		Inguinal	0.15 ± 0.13	0.10	0.03 - 0.45	
Diphenhydramine	14	Femoral	3165.5 ± 6072.5	369.0	56.5 - 19733.0	.212
		Iliac	3304.7 ± 6450.0	269.0	53.3 - 18468.0	
		Inguinal	2441.1 ± 4549.1	326.0	0.0 - 12761.0	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Citalopram	14	Femoral	356.2 ± 534.1	148.5	10.5 - 1742.0	.306
		Iliac	444.3 ± 591.5	202.5	34.0 - 1960.0	
		Inguinal	269.5 ± 324.0	175.0	24.1 - 1229.0	
THC	14	Femoral	3.4 ± 3.4	1.8	1.0 - 12.6	.241
		Iliac	1.5 ± 1.4	1.4	0.0 - 4.2	
		Inguinal	1.8 ± 4.2	0.0	0.0 - 15.3	
Methadone	13	Femoral	363.2 ± 216.7	341.0	33.0 - 849.0	.662
		Iliac	344.1 ± 198.7	308.0	30.0 - 653.0	
		Inguinal	338.9 ± 226.1	271.0	25.0 - 885.0	
THC-COOH	12	Femoral	23.0 ± 19.6	15.2	5.0 - 64.9	***.021***
		Iliac	20.0 ± 14.8	16.9	4.6 - 50.4	
		Inguinal	15.0 ± 11.2	11.7	4.1 - 45.3	
Hydrocodone	12	Femoral	372.7 ± 992.8	35.6	13.1 - 3512.0	.349
		Iliac	236.1 ± 454.8	43.5	17.9 -1639.0	
		Inguinal	150.1 ± 293.7	35.5	15.8 - 1062.0	
Nordiazepam	12	Femoral	341.9 ± 236.4	286.0	62.0 -688.0	.981
		Iliac	360.9 ± 211.0	336.0	68.4 -732.0	
		Inguinal	357.3 ± 202.0	319.0	93.6 - 613.0	
Fluoxetine	11	Femoral	385.1 ± 420.6	189.0	24.8 - 1431.0	.121
		Iliac	168.1 ± 197.7	98.6	0.0 - 562.0	
		Inguinal	88.9 ± 116.2	0.0	0.0 - 270.0	
Diazepam	10	Femoral	287.1 ± 182.3	303.0	59.8 - 640.0	.386
		Iliac	267.9 ± 157.5	295.0	0.0 - 532.0	
		Inguinal	288.5 ± 168.3	301.5	0.0 - 627.0	
Nortriptyline	10	Femoral	245.8 ± 149.7	253.0	41.4 - 478.0	.065
		Iliac	185.0 ± 187.3	81.3	0.0 - 493.0	
		Inguinal	149.5 ± 131.1	116.2	0.0 - 339.0	
Amitriptyline	9	Femoral	1206.6 ± 1826.6	357.0	23.3 - 5324.0	.597
		Iliac	850.4 ± 1494.3	316.0	20.3 - 4749.0	
		Inguinal	744.6 ± 1257.4	316.0	0.0 - 4000.0	
Fentanyl	9	Femoral	8.8 ± 5.9	7.1	1.9 - 21.1	.714
		Iliac	7.3 ± 7.1	5.7	0.0 - 23.9	
		Inguinal	8.4 ± 4.7	10.1	1.7 - 13.9	
EDDP	9	Femoral	51.2 ± 27.0	51.0	27.8 - 116.0	***.001***
		Iliac	50.5 ± 26.6	46.8	25.4 - 113.0	
		Inguinal	34.7 ± 24.5	37.1	0.0 - 74.9	
Acetaminophen	8	Femoral	90.3 ± 130.2	36.3	4.3 - 395.0	.271
(mg/L)		Iliac	84.3 ± 105.2	38.7	4.9 - 308.0	
		Inguinal	63.6 ± 70.4	33.1	3.5 - 213.0	
Gabapentin	8	Femoral	22.9 ± 20.4	19.3	1.6 -56.30	.528
(mg/L)		Iliac	26.6 ± 25.1	23.7	2.1 - 75.8	
		Inguinal	21.6 ± 15.0	24.2	2.1 - 39.3	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Nortramadol	8	Femoral	383.4 ± 318.5	270.5	139.0 -1081.0	.074
		Iliac	493.5 ± 426.0	391.5	151.0 - 1434.0	
		Inguinal	452.1 ± 303.8	390.0	146.0 - 1122.0	
Paroxetine	7	Femoral	615.1 ± 938.3	400.0	27.4 - 2695.0	.395
		Iliac	679.0 ± 1149.4	401.0	32.6 - 3254.0	
		Inguinal	346.4 ± 329.0	242.0	31.6 - 907.0	
Tramadol	7	Femoral	3281.6 ± 4370.2	1092.0	280.0 - 11869.0	.528
		Iliac	3151.0 ± 4125.1	1296.0	0.0 - 11429.0	
		Inguinal	2880.1 ± 3297.6	2091.0	0.0 - 9013.0	
Hydromorphone	7	Femoral	11.7 ± 10.5	5.1	2.6 -25.3	.502
		Iliac	11.2± 11.0	4.4	0.0 - 25.6	
		Inguinal	13.6 ± 12.1	18.1	0.0 - 30.2	
Methamphetamine	7	Femoral	442.7 ± 514.7	170.0	71.0 -1253.0	.117
		Iliac	572.0 ± 721.9	177.0	75.3 -1745.0	
		Inguinal	412.7 ± 477.5	160.0	83.8 -1242.0	
Norfluoxetine	6	Femoral	254.2 ± 119.4	239.5	121.0 -441.0	.135
		Iliac	157.0 ± 102.7	126.3	39.5 - 284.0	
		Inguinal	123.5 ± 141.2	81.6	0.0 - 343.0	
Quetiapine	6	Femoral	260.1 ± 153.1	258.0	90.6 - 480.0	.308
		Iliac	705.4 ± 954.6	429.0	77.5 - 2613.0	
		Inguinal	442.3 ± 413.3	354.5	62.1 - 1180.0	
Amlodipine	6	Femoral	187.8 ± 404.4	24.8	9.9 - 1013.0	.372
		Iliac	319.2 ± 699.7	37.0	0.0 - 1746.0	
		Inguinal	177.0 ± 359.7	35.9	7.0 - 910.0	
Cyclobenzaprine	6	Femoral	146.8 ± 133.6	86.6	33.6 - 375.0	.545
		Iliac	148.2 ± 177.7	70.9	30.7 - 490.0	
		Inguinal	168.9 ± 179.7	103.9	47.9 - 523.0	
Lamotrigine	6	Femoral	3.0 ± 1.2	3.1	1.4 - 4.5	.133
(mg/L)		Iliac	2.6 ± 0.7	2.6	1.5 - 3.4	
		Inguinal	2.2 ± 0.9	2.0	1.4 - 3.6	
Lorazepam	6	Femoral	62.7 ± 24.9	55.6	33.4 - 93.1	.804
		Iliac	60.2 ± 29.6	62.4	19.6 - 103.0	
		Inguinal	70.1 ± 45.3	58.0	22.3 - 140.0	
Metoprolol	6	Femoral	153.5 ± 127.9	105.9	42.2 - 353.0	.457
		Iliac	171.2 ± 133.4	133.8	57.4 - 362.0	
		Inguinal	150.9 ± 112.5	107.9	56.1 - 313.0	
Mirtazapine	6	Femoral	118.2 ± 85.5	120.5	26.1 - 226.0	.311
		Iliac	109.4 ± 100.2	83.7	28.7 - 284.0	
		Inguinal	135.3 ± 115.8	124.0	29.1 - 276.0	
Pregabalin	6	Femoral	10.0 ± 11.8	6.7	2.1 -33.7	.994
(mg/L)		Iliac	10.1 ± 14.8	5.8	0.6 - 39.9	
		Inguinal	10.0 ± 14.3	5.8	0.6 - 38.8	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Temazepam	6	Femoral	233.8 ± 145.0	205.0	74.8 - 411.0	.878
		Iliac	239.0 ± 168.8	193.5	75.2 -486.0	
		Inguinal	253.3 ± 160.7	278.5	62.0 - 408.0	
Zolpidem	6	Femoral	451.9 ± 700.3	116.1	24.8 - 1819.0	.275
		Iliac	512.3 ± 904.5	121.0	21.6 - 2336.0	
		Inguinal	367.4 ± 653.5	69.6	32.4 - 1684.0	
Amphetamine	5	Femoral	192.5 ± 103.8	160.0	65.4 - 303.0	***.021***
		Iliac	236.7 ± 137.6	233.0	66.4 - 438.0	
		Inguinal	155.1 ± 119.9	147.0	0.0 - 291.0	
Carbamazepine	5	Femoral	12.8 ± 10.2	10.3	2.9 - 30.1	.454
(mg/L)		Iliac	12.8 ± 11.1	9.9	2.7 - 31.6	
		Inguinal	14.8 ± 15.2	9.3	2.5 - 41.1	
Duloxetine	5	Femoral	149.9 ± 163.6	66.8	54.6 - 437.0	.348
		Iliac	173.6 ± 244.0	65.7	57.6 - 610.0	
		Inguinal	110.8 ± 139.4	65.9	0.0 - 354.0	
Norsertraline	5	Femoral	932.6 ± 1067.4	373.0	162.0 - 2750.0	.109
		Iliac	112.6 ± 163.9	0.0	0.0 - 360.0	
		Inguinal	94.0 ± 134.7	0.0	0.0 - 291.0	
Sertraline	5	Femoral	1036.8 ± 1482.5	201.0	133.0 - 3577.0	.112
		Iliac	367.4 ± 821.5	0.0	0.0 - 1837.0	
		Inguinal	279.2 ± 454.3	139.0	0.0 - 1079.0	
Bupropion	4	Femoral	367.0 ± 421.5	163.0	143.0 - 999.0	.462
		Iliac	388.0 ± 490.0	145.5	138.0 - 1123.0	
		Inguinal	420.3 ± 521.8	183.0	116.0 - 1199.0	
Dextromethorphan	4	Femoral	78.1 ± 87.1	44.7	16.2 - 207.0	.150
		Iliac	99.4 ± 110.8	54.0	25.8 - 264.0	
		Inguinal	75.5 ± 86.9	51.1	0.0 - 200.0	
Promethazine	3	Femoral	116.7 ± 110.3	82.9	27.3 - 240.0	.142
		Iliac	73.3 ± 127.0	0.0	0.0 - 220.0	
		Inguinal	79.3 ± 137.4	0.0	0.0 - 238.0	
Benzoylecgonine	3	Femoral	358.0 ± 169.7	381.0	178.0 - 515.0	.793
		Iliac	350.7 ± 125.3	419.0	206.0 - 427.0	
		Inguinal	333.3 ± 118.9	401.0	196.0 - 403.0	
Buprenorphine	3	Femoral	2.7 ± 2.2	2.9	0.5 - 4.8	.067
		Iliac	2.3 ± 2.2	2.6	0.0 - 4.3	
		Inguinal	0.6 ± 0.5	0.8	0.0 - 0.9	
Carbamazepine	3	Femoral	3.3 ± 3.2	1.7	1.2 - 7.0	.423
Epoxide (mg/L)		Iliac	3.2 ± 2.8	2.2	1.0 - 6.4	
		Inguinal	4.3 ± 4.7	1.9	1.3 - 9.8	
Clonazepam	3	Femoral	5.8 ± 0.8	5.6	5.1 - 6.7	.655
		Iliac	6.0 ± 1.0	6.0	5.0 - 7.0	
		Inguinal	6.1 ± 0.5	6.0	5.7 - 6.7	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Codeine	3	Femoral	73.8 ± 83.3	27.6	23.8 - 170.0	.434
		Iliac	60.3 ± 56.1	28.8	27.0 - 125.0	
		Inguinal	56.5 ± 57.6	25.5	21.1 - 123.0	
Meprobamate	3	Femoral	20.6 ± 24.2	10.6	3.1 - 48.3	.507
(mg/L)		Iliac	19.0 ± 20.9	10.5	3.7 - 42.9	
		Inguinal	18.4 ± 20.0	9.7	4.2 - 41.3	
Norvenlafaxine	3	Femoral	373.2 ± 306.7	352.0	77.6 - 690.0	.330
		Iliac	557.5 ± 556.3	425.0	79.4 - 1168.0	
		Inguinal	442.8 ± 397.3	422.0	56.3 -850.0	
Oxymorphone	3	Femoral	35.6 ± 12.6	32.0	25.2 - 49.6	.496
		Iliac	45.9 ± 49.5	19.8	14.8 - 103.0	
		Inguinal	25.3 ± 33.8	12.2	0.0 - 63.7	
Pseudoephedrine	3	Femoral	317.7 ± 253.4	222.0	126.0 - 605.0	.734
		Iliac	295.3 ± 208.8	188.0	162.0 - 536.0	
		Inguinal	301.3 ± 268.7	182.0	113.0 - 609.0	
Valproic Acid	3	Femoral	27.6 ± 4.1	26.9	23.8 - 32.0	.950
(mg/L)		Iliac	28.6 ± 5.4	30.3	22.5 - 32.9	
		Inguinal	27.9 ± 5.8	28.6	21.8 - 33.3	
Carbon Monoxide	2	Femoral	8.0 ± 2.6	9.8	6.1 - 9.8	.444
(% sat)		Iliac	2.9 ± 4.1	0.0	0.0 - 5.8	
		Inguinal	2.7 ± 3.7	0.0	0.0 - 5.3	
Levetiracetam	2	Femoral	339.3 ± 457.8	339.3	15.6 - 663.0	.588
(mg/L)		Iliac	307.4 ± 403.9	307.4	21.8 - 593.0	
		Inguinal	308.3 ± 405.5	308.3	21.6 - 595.0	
6-acetylmorphine	2	Femoral	26.3 ± 1.9	26.3	24.9 - 27.6	.617
		Iliac	12.8 ± 18.0	12.8	0.0 - 25.5	
		Inguinal	20.8 ± 5.9	20.8	16.6 - 24.9	
Lidocaine	2	Femoral	2.1 ± 0.5	2.1	1.7 - 2.4	.372
		Iliac	1.9 ± 0.1	1.9	1.8 - 1.9	
		Inguinal	1.0 ± 0.8	1.0	0.4 - 1.5	
Norbuprenorphine	2	Femoral	5.6 ± 4.6	5.6	2.3 - 8.8	.067
		Iliac	6.0 ± 5.3	6.0	2.2 - 9.7	
		Inguinal	2.9 ± 4.0	2.9	0.0 - 5.7	
Oxazepam	2	Femoral	131.7 ± 96.7	131.7	63.3 - 200.0	.421
		Iliac	109.9 ± 68.1	109.9	61.7 - 158.0	
		Inguinal	109.5 ± 72.8	109.5	58.0 - 161.0	
Trazodone	2	Femoral	0.4 ± 0.0	0.4	0.3 - 0.4	.524
		Iliac	0.2 ± 0.2	0.2	0.0 - 0.4	
		Inguinal	0.3 ± 0.0	0.3	0.3 - 0.3	
Warfarin	2	Femoral	0.5 ± 0.1	0.5	0.4 - 0.5	.250
(mg/L)		Iliac	0.7 ± 0.3	0.7	0.5 - 0.9	
		Inguinal	0.8 ± 0.4	0.8	0.6 - 1.1	

Table 2: Summary of drugs and/or metabolite concentrations (ng/mL) in only one specimen triplet. Femoral blood was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood was collected by direct visualization and clamping the vein. Inguinal blood was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	FEMORAL	ILIAC	INGUINAL
Alpha-PVP*	401.0	502.0	480.0
Carisoprodol (mg/L)	13.0	10.5	9.1
Chlordiazepoxide	644.0	660.0	837.0
Clomipramine	224.0	422.0	448.0
Clozapine	725.0	818.0	729.0
Doxepin	5899.0	4527.0	3904.0
Doxylamine	324.0	205.0	166.0
Methylphenidate	54.8	44.0	28.9
Midazolam	77.1	0.0	0.0
Norclomipramine	708.0	2546.0	2403.0
Norclozapine	242.0	262.0	215.0
Nordoxepin	598.0	655.0	577.0
Rocuronium	1013.0	0.0	0.0
Venlafaxine	1180.0	1334.0	1383.0
Demoxepam	185.0	145.0	192.0
Hydroxyzine	65.2	59.9	31.4

*Alpha-pyrrolidinopentiophenone

There was large variability in drug concentrations within and between subjects. In 49 specimen triplets, the femoral blood specimen (Specimen 1) was positive while the iliac (Specimen 2), inguinal (Specimen 3) or both were negative (Table 3). This occurred for THC (*n = 9*), fluoxetine (*n = 4*), norsertraline (*n = 4*) hydromorphone (*n = 3*), norfluoxetine (*n = 2*), nortriptyline (*n = 2*), oxycodone (*n = 2*), promethazine (*n = 2*), sertraline (*n = 2*), and on a single specimen triplet for multiple other drugs (Table 3).

Table 3: Summary of drug/metabolite concentrations (ng/mL) found in only one or two of the specimens. Femoral blood was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood was collected by direct visualization and clamping the vein. Inguinal blood was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	FEMORAL	ILIAC	INGUINAL
THC	12.6	3.7	0
THC	2.6	0	0
THC	1.8	0	0
THC	1.2	0	0
THC	1.8	3.1	0
THC	3.4	1.3	0
THC	1.2	0	0
THC	3.1	0	0
THC	8.6	2.5	0
Sertraline	135	0	0
Sertraline	201	0	139
Sertraline	1138	0	178
Sertraline	133	0	0
Norsertraline	351	0	0
Norsertraline	1027	360	0
Norsertraline	2750	0	179
Norsertraline	162	0	0
Fluoxetine	111	0	0
Fluoxetine	308	244	0
Fluoxetine	24.8	0	0
Fluoxetine	65.1	64.3	0
Hydromorphone	5.1	0	18.1
Hydromorphone	3.1	4.4	0
Hydromorphone	2.6	2.6	0
EDDP	32.4	25.4	0
EDDP	27.8	30.8	0
Norfluoxetine	281	39.5	0
Norfluoxetine	121	95.5	0
Nortriptyline	228	0	0
Nortriptyline	41.4	20.3	0
Oxycodone	15.1	14.4	0
Oxycodone	10.1	11.3	0
Promethazine	82.9	0	0
Promethazine	27.3	0	0
6-acetylmorphine	24.9	0	24.9
7-aminoclonazepam	12.5	0	18.5
Amitriptyline	23.3	20.3	0
Amlodipine	26.6	0	13.4
Amphetamine	65.4	66.4	0
Buprenorphine	0.5	0	0
Carbon Monoxide (% sat)	9.8	0	0
Dextromethorphan	16.2	25.8	0
Diazepam	59.8	0	0
Diphenhydramine	56.5	53.3	0
Duloxetine	133	57.6	0
Fentanyl	5.3	0	10.1
Midazolam	77.1	0	0

DRUG/METABOLITE	FEMORAL	ILIAC	INGUINAL
Tramadol	284	0	0
Trazodone	0.33	0	0.29

Within subject variability was characterized by calculating concentration ratios. We found large variability in iliac/femoral, inguinal/femoral and iliac/inguinal ratios, although concentrations between the three sites within a specimen triplet were generally very similar (Table 4). The mean ratios among all drug/metabolites were 1.0, 0.9 and 1.2 for iliac/femoral, inguinal/femoral and iliac/inguinal comparisons, respectively. The lowest ratio was 0.0 (one specimen was negative), found in multiple specimen triplets (see Table 3) and the highest ratio was 10.9 found when comparing inguinal/femoral blood positive for THC.

Table 4: Summary of drug/metabolite mean concentration ratios (range). Femoral blood (FEM) was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood (ILIAC) was collected by direct visualization and clamping the vein. Inguinal blood (ING) was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	N	ILIAC/FEM	ING/FEM	ILIAC/ING
6-acetylmorphine	2	0.5 (0.0 - 0.9)	0.8 (0.6 - 1.0)	0.8 (0.0 - 1.5)
7-aminoclonazepam	21	1.6 (0.2 - 3.9)	1.8 (0.2 - 4.5)	1.0 (0.6 - 3.0)
Acetaminophen	8	1.1 (0.8 - 1.7)	0.9 (0.5 - 1.3)	1.2 (0.8 - 1.7)
Alpha-PVP	1	1.3 (1.3 - 1.3)	1.2 (1.2 - 1.2)	1.0 (1.0 - 1.0)
Alprazolam	37	1.0 (0.4 - 1.6)	1.1 (0.6 - 2.2)	1.0 (0.4 - 1.8)
Amitriptyline	9	0.9 (0.3 - 1.2)	0.8 (0.0 - 1.4)	1.5 (0.2 - 5.1)
Amlodipine	6	2.2 (0.0 - 8.8)	1.7 (0.5 - 6.1)	1.1 (0.0 - 1.9)
Amphetamine	5	1.2 (0.9 - 1.7)	0.7 (0.0 - 1.1)	1.6 (1.1 - 2.0)
Benzoylecgonine	3	1.0 (0.8 - 1.2)	1.0 (0.8 - 1.1)	1.1 (1.0 - 1.1)
Buprenorphine	3	0.6 (0.0 - 0.9)	0.2 (0.0 - 0.3)	4.1 (2.9 - 5.4)
Bupropion	4	1.0 (0.9 - 1.1)	1.1 (0.8 - 1.4)	1.0 (0.6 - 1.3)
Carbamazepine	5	1.0 (0.8 - 1.0)	1.0 (0.9 - 1.4)	1.0 (0.8 - 1.1)
Carbamazepine Epoxide	3	1.0 (0.8 - 1.3)	1.2 (1.1 - 1.4)	0.9 (0.7 - 1.2)
Carbon Monoxide	2	0.5 (0.0 - 1.0)	0.4 (0.0 - 0.9)	1.1 (1.1 - 1.1)
Carisoprodol	1	0.8 (0.8 - 0.8)	0.7 (0.7 - 0.7)	1.2 (1.2 - 1.2)
Chlordiazepoxide	1	1.0 (1.0 - 1.0)	1.3 (1.3 - 1.3)	0.8 (0.8 - 0.8)
Citalopram	14	2.1 (0.6 - 7.8)	1.2 (0.2 - 2.8)	1.6 (0.8 - 3.6)
Clomipramine	1	1.9 (1.9 - 1.9)	2.0 (2.0 - 2.0)	0.9 (0.9 - 0.9)
Clonazepam	3	1.0 (0.9 - 1.2)	1.1 (1.0 - 1.2)	1.0 (0.9 - 1.0)
Clozapine	1	1.1 (1.1 - 1.1)	1.0 (1.0 - 1.0)	1.1 (1.1 - 1.1)
Codeine	3	1.0 (0.7 - 1.2)	0.9 (0.7 - 1.1)	1.1 (1.0 - 1.3)
Cyclobenzaprine	6	0.9 (0.6 - 1.3)	1.2 (0.7 - 1.4)	0.8 (0.6 - 1.1)
Demoxepam	1	0.8 (0.8 - 0.8)	1.0 (1.0 - 1.0)	0.8 (0.8 - 0.8)
Dextromethorphan	4	1.3 (1.1 - 1.6)	0.8 (0.0 - 1.2)	1.2 (1.0 - 1.3)
Diazepam	10	0.9 (0.0 - 1.4)	1.0 (0.0 - 1.7)	0.9 (0.7 - 1.1)
Diphenhydramine	14	0.8 (0.4 - 1.5)	0.8 (0.0 - 1.1)	1.0 (0.3 - 1.5)

DRUG/METABOLITE	N	ILIAC/FEM	ING/FEM	ILIAC/ING
Doxepin	1	0.8 (0.8 - 0.8)	0.7 (0.7 - 0.7)	1.2 (1.2 - 1.2)
Doxylamine	1	0.6 (0.6 - 0.6)	0.5 (0.5 - 0.5)	1.2 (1.2 - 1.2)
Duloxetine	5	1.0 (0.4 - 1.4)	0.8 (0.0 - 1.2)	1.2 (0.9 - 1.7)
EDDP	9	1.0 (0.8 - 1.4)	0.6 (0.0 - 1.0)	1.3 (1.0 - 1.5)
Ethanol	15	1.0 (0.8 - 1.4)	1.0 (0.9 - 1.1)	1.0 (0.8 - 1.4)
Fentanyl	9	0.9 (0.0 - 1.3)	1.1 (0.7 - 1.9)	0.9 (0.0 - 1.7)
Fluoxetine	7	0.7 (0.0 - 1.1)	0.4 (0.0 - 1.5)	1.4 (0.7 - 2.8)
Gabapentin	8	1.1 (0.7 - 1.7)	1.1 (0.6 - 1.9)	1.1 (0.5 - 2.0)
Hydrocodone	12	1.2 (0.5 - 2.1)	0.9 (0.3 - 1.5)	1.4 (0.9 - 2.5)
Hydromorphone	7	0.9 (0.0 - 1.4)	1.2 (0.0 - 3.5)	0.7 (0.0 - 1.1)
Hydroxyzine	1	0.9 (0.9 - 0.9)	0.5 (0.5 - 0.5)	1.9 (1.9 - 1.9)
Lamotrigine	6	0.9 (0.6 - 1.3)	0.8 (0.4 - 1.0)	1.3 (0.7 - 2.4)
Levetiracetam	2	1.1 (0.9 - 1.4)	1.1 (0.9 - 1.4)	1.0 (1.0 - 1.0)
Lidocaine	2	0.9 (0.8 - 1.1)	0.5 (0.2 - 0.9)	2.9 (1.3 - 4.5)
Lorazepam	6	0.9 (0.6 -1.2)	0.8 (0.0 - 1.5)	1.0 (0.7 - 1.3)
Meprobamate	3	1.0 (0.9 -1.2)	1.0 (0.9 - 1.4)	1.0 (0.9 - 1.1)
Methadone	13	1.0 (0.5 - 1.8)	0.9 (0.6 - 1.5)	1.1 (0.5 - 1.7)
Methamphetamine	7	1.2 (0.9 - 1.5)	1.1 (0.5 - 1.8)	1.2 (0.6 - 1.9)
Methylphenidate	1	0.8 (0.8 - 0.8)	0.5 (0.5 - 0.5)	1.5 (1.5 - 1.5)
Metoprolol	6	1.1 (1.0 - 1.4)	1.1 (0.7 - 1.5)	1.1 (0.9 - 1.4)
Midazolam	1	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
Mirtazapine	6	0.9 (0.5 - 1.4)	1.0 (0.4 - 1.3)	0.9 (0.6 - 1.1)
Morphine	19	1.2 (0.4 - 3.9)	1.1 (0.4 - 3.0)	1.5 (0.2 - 4.7)
Norbuprenorphine	2	1.0 (1.0 - 1.1)	0.3 (0.0 - 0.6)	1.4 (1.0 - 1.7)
Norclomipramine	1	3.6 (3.6 - 3.6)	3.4 (3.4 - 3.4)	1.1 (1.1 - 1.1)
Norclozapine	1	1.1 (1.1 - 1.1)	0.9 (0.9 - 0.9)	1.2 (1.2 - 1.2)
Nordiazepam	11	1.1 (0.7 - 1.7)	1.1 (0.7 - 1.9)	1.0 (0.7 - 1.2)
Nordoxepin	1	1.1 (1.1 - 1.1)	1.0 (1.0 - 1.0)	1.1 (1.1 - 1.1)
Norfluoxetine	6	0.7 (0.1 - 1.4)	0.6 (0.0 - 1.7)	2.8 (0.7 - 9.1)
Norsertraline	5	0.2 (0.0 - 0.5)	0.2 (0.0 - 0.8)	0.3 (0.0 - 0.7)
Nortramadol	8	1.3 (0.8 - 2.0)	1.3 (1.0 - 2.1)	1.0 (0.4 - 1.3)
Nortriptyline	10	0.7 (0.0 - 1.3)	0.6 (0.0 - 1.2)	1.3 (0.2 - 3.0)
Norvenlafaxine	3	1.3 (1.0 - 1.7)	1.1 (0.7 - 1.2)	1.3 (1.0 - 1.4)
Oxazepam	2	0.9 (0.8 - 1.0)	0.9 (0.8 - 0.9)	1.0 (1.0 - 1.1)
Oxycodone	16	1.1 (0.7 - 1.8)	0.9 (0.0 - 1.4)	1.1 (0.7 - 1.8)
Oxymorphone	3	1.1 (0.6 - 2.1)	0.6 (0.0 - 1.3)	1.4 (1.2 - 1.6)
Paroxetine	7	1.0 (0.8 - 1.2)	0.8 (0.3 - 1.2)	1.5 (0.7 - 3.6)
Pregabalin	6	1.0 (0.1 - 1.8)	1.0 (0.1 - 1.6)	1.0 (0.9 - 1.1)
Promethazine	3	0.3 (0.0 - 0.9)	0.3 (0.0 - 1.0)	0.9 (0.9 - 0.9)
Pseudoephedrine	3	1.0 (0.8 - 1.3)	0.9 (0.8 - 1.0)	1.1 (0.9 - 1.4)
Quetiapine	6	2.6 (0.9 - 9.2)	1.7 (0.7 - 4.1)	1.4 (0.9 - 2.2)
Rocuronium	1	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
Sertraline	5	0.1 (0.0 - 0.5)	0.2 (0.0 - 0.7)	0.6 (0.0 - 1.7)
Temazepam	6	1.0 (0.8 - 1.2)	1.1 (0.8 - 2.3)	1.0 (0.4 - 1.3)
THC	14	0.6 (0.0 - 1.7)	1.1 (0.0 - 10.9)	0.8 (0.1 - 1.3)
THC-COOH	12	1.0 (0.5 - 2.2)	0.8 (0.4 - 2.0)	1.3 (0.7 - 2.2)
Tramadol	7	0.9 (0.0 - 1.3)	1.0 (0.0 - 1.9)	1.0 (0.6 - 1.3)
Trazodone	2	0.4 (0.0 - 0.9)	0.8 (0.7 - 0.9)	0.6 (0.0 - 1.3)
Valproic Acid	3	1.1 (0.8 - 1.4)	1.0 (0.8 - 1.2)	1.0 (0.9 - 1.2)
Venlafaxine	1	1.1 (1.1 - 1.1)	1.2 (1.2 - 1.2)	1.0 (1.0 - 1.0)
Warfarin	2	1.4 (1.1 - 1.6)	1.7 (1.4 - 2.0)	0.8 (0.8 - 0.8)
Zolpidem	6	1.0 (0.7 - 1.6)	0.8 (0.4 - 1.3)	1.4 (0.7 - 1.8)

4. CONCLUSIONS

4.1 Statistical Significance

Reliable interpretation of postmortem blood drug concentrations depends greatly on pre-analytical variables such as collection site and possible analyte instability. Blood collected from any peripheral site, regardless of collection method, is often considered the most reliable specimen when interpreting toxicological findings. We found no statistical difference between the three collection/shipping methods for most drugs. This finding is not surprising considering the small sample size within each drug/metabolite group and the large variability in drug concentration within each specimen collection/shipping procedure. For three analytes, however, statistical differences were detected. Methadone metabolite (2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine, EDDP), delta-9-carboxy-THC and amphetamine concentrations in the inguinal specimen were significantly lower than in the other two sites. Meaningful conclusions based on these findings remain suspect. While statistically significant differences were detected, small sample size should be considered. Nevertheless, one possible explanation may include analyte instability in inguinal blood after a single freeze/thaw cycle. The inguinal specimen was collected from approximately the same site and immediately following the femoral blood specimen. The only differences between these two collections is that, while taken from the same general location in the inguinal region, each was obtained by a different blind stick of the femoral vein. The femoral blood (Specimen 1) was temporarily refrigerated prior to submission to the laboratory and was shipped at ambient temperature shortly after autopsy. The inguinal specimen (Specimen 3), was immediately frozen at -57°C. Several weeks passed in some cases before informed consent was obtained from next-of-kin. Laboratory results on the femoral specimen were received within approximately week. No significant decrease in EDDP

concentration was found in -20°C stored breast milk after one month (Nikolaou, Papoutsis et al. 2008); however, EDDP was found to be unstable in oral fluid stored refrigerated for two months (Fucci and De Giovanni 2008). Conversion of methadone to EDDP occurs by demethylation *in vivo* (Danielson, Mozayani et al. 2008); however, we are unaware of studies demonstrating this conversion spontaneously in authentic specimens or fortified samples.

Delta-9-carboxy-THC concentrations may change when stored at various conditions after collection. In a recent study, whole blood specimens were collected from cannabis users after controlled drug administration and pooled (Scheidweiler, Schwope et al. 2013). Samples were stored at room temperature, refrigerated (4°C) and frozen (-20°C) for various durations. Delta-9-carboxy-THC concentrations increased when stored at room temperature and remained stable for 4 an 26 weeks when stored refrigerated and frozen, respectively. This may be explained by conversion of delta-9-carboxy-THC-glucuronide to delta-9-carboxy-THC at higher temperature, which also was demonstrated in authentic urine specimens (Skopp and Potsch 2004). The authors also evaluated delta-9-carboxy-THC-glucuronide instability in fortified samples at various pH. In that study, the magnitude of decrease and simultaneous increase in delta-9-carboxy-THC was pH dependent and occurred even at pH 5. Therefore, our data appear to be consistent with an increase in delta-9-caboxy-THC when collected in the femoral vein and shipped at ambient temperature, instead of its decrease in the other two specimens maintained frozen.

As stated above, statistical conclusions based on a small sample size remain suspect; however, significantly lower amphetamine concentration in the inguinal specimen may be explained by site of collection and/or PMR. Amphetamine appears to be stable at room temperature, with moderate decreases over three months (Giorgi and Meeker 1995). The short period in which the femoral blood was shipped at ambient temperature should not cause

significant amphetamine concentration decreases. Post Hoc analysis revealed that the inguinal blood amphetamine concentration was significantly lower than the iliac blood concentration. Both of these specimens were collected and shipped to the laboratory after frozen storage. Given its relative stability, this finding suggests possible PMR into iliac site. The iliac vein found within the abdominal cavity and some drugs may re-distribute into the iliac blood to a greater extent than more distal sites such as femoral blood. Subclavian blood was recently evaluated to determine whether drug concentrations were closer to peripheral or heart blood concentrations (Molina and Hargrove 2013). The authors found that generally, drug concentrations were lower in subclavian blood than in heart blood, but higher than in peripheral blood and recommended the specific site be included in postmortem toxicology evaluation.

Trends toward significance ($p < 0.10$) were determined for nortiptyline ($n = 10$, $p = 0.065$), N-desmethyltramadol ($n = 8$, $p = 0.074$), buprenorphine ($n = 3$, $p = 0.067$) and norbuprenorphine ($n = 2$, $p = 0.067$). As with the aforementioned analytes, different drug concentrations for nortriptyline, N-desmtehyltramadol, buprenorphine and norbuprenorphine, may be explained by analyte instability or potential PMR within the peripheral compartment. Nortriptyline appears to be stable in plasma (Hotha, Ravindranath et al. 2010) and should not significantly degrade at ambient or frozen temperature. Buprenorphine and norbuprenorphine in whole blood also appear to be stable at -20°C (Seldén, Roman et al. 2011). To our knowledge, postmortem conversion from amitriptyline to nortriptyline or buprenorphine to norbuprenorphine has not been shown. These data demonstrate potential site dependent or pre-analytical changes in drug concentrations for these analytes.

4.2 Postmortem redistribution

Postmortem redistribution of drugs is widely published in the literature. Several reviews have been published addressing topics such as interpretation of drug levels and pharmacokinetic relationships (Ferner 2008), the relationship between the putrefactive process and movement of drugs into different compartments (Pelissier-Alicot, Gaulier et al. 2003) and estimating ante-mortem drug concentrations from autopsy specimens (Cook, Braithwaite et al. 2000). Multiple others have investigated PMR of individual drugs. The mechanisms of PMR are complex and involve movement of drugs along concentration gradients from various organs, changes in blood properties such as putrefaction and multiple chemical properties of the individual drug, such as pK_a and lipophilicity (Pelissier-Alicot, Gaulier et al. 2003). Additionally, putrefaction and the influence of bacteria may alter drug concentrations in the postmortem interval or after specimen collection (Butzbach 2010). These reviews help to explain the complexity of PMR and the difficulty it poses in drug related deaths.

We found multiple specimen triplets in which iliac or inguinal or both blood specimens were negative. Cases were selected based in part on whether femoral blood (Specimen 1) was positive for one or more drugs/metabolites. Drug concentrations in the remaining two specimens were assumed to be positive, at least at the time of autopsy. Negative findings in these specimens suggests potential degradation of analytes in the frozen specimens (Specimens 2 and 3), or artificially elevated concentration in the initial specimen, owing to PMR. Given the large variability in analyte concentrations, it also is possible that some drugs/metabolites were below the analytical cutoff in one or two specimens due to an overall low venous blood concentration. Low analyte concentrations near the cutoff were found for THC, hydromorphone and buprenorphine (see Table 3); however, the remaining analytes listed in Table 3 were well within

the detectable range. THC concentrations decreased significantly in the two frozen specimens, likely owing to adherence to container surfaces. This phenomenon has been documented previously with THC losses of 60 to 100% when specimens were stored in polystyrene tubes for one month (Christophersen 1986).

Another explanation may be PMR. The classical method of characterizing PMR is by comparing heart/peripheral blood drug/metabolite concentrations. A heart/peripheral blood ratio greater or less than 1 suggests potential re-distribution of drugs within these sites. Peripheral blood drug/metabolite concentration is believed to be more stable after death than heart blood due to re-distribution from multiple potential tissue sites within the central cavity. Few studies have examined potential re-distribution within the peripheral compartment. One study demonstrated re-distribution of fentanyl by comparing fentanyl concentrations in femoral blood collected shortly after death to a second specimen collected at autopsy (Olson, Luckenbill et al.). The mean concentrations for the first and second femoral blood specimens were 4.6 ng/mL to 17.6 ng/mL, respectively. In all cases, the concentration increased in the second specimen.

We examined concentration ratios between the three specimens to demonstrate within subject variability in analyte concentration (Table 4). Generally, concentrations were similar between the three specimens; however, some ratios were less than 0.5 or greater than 2.0. This indicates that pre-analytical factors influence drug concentration and potentially toxicological interpretation. Therefore, while generally it appears that any of these collection/shipping procedures should yield similar results, there still is the potential for misinterpretation based on peripheral blood concentration. For example, an increase in concentration due to PMR, from within the therapeutic to within the toxic range, could yield an incorrect interpretation of toxicity.

Usually, multiple specimens are collected during autopsy for toxicological determination. Heart blood, peripheral blood, urine, vitreous fluid, liver and other organ tissues may be collected. Drug/metabolite concentrations in peripheral blood remain an essential part of the overall toxicological investigation; however, concentrations in other specimen types also aid interpretation. These data further support the practice of basing interpretation on the totality of evidence, including examination of peripheral blood and other specimen types, as well as other investigative factors.

To our knowledge, there are no other prospective studies that have compared drug/metabolite concentrations in multiple peripheral blood sites. The total scope of this study, number of different drugs/metabolites detected and total cases has not been reported elsewhere. These data will be valuable for toxicologists, pathologists and clinicians in interpreting drug/metabolite concentrations amid other case findings.

5. STUDY LIMITATIONS

The study was designed with parameters that were very specific in an effort to maximize the value of the data generated; however, these data are limited and should be applied only to applicable situations. Unlike many studies dealing with PMR, we did not compare differences in drug concentrations between peripherally and centrally obtained blood specimens. Instead, we concentrated solely on possible differences in analyte concentrations in peripheral sites, limited to femoral and iliac venous blood specimens, which are commonly collected during autopsy. PMR is an accepted phenomenon and studies are found widely throughout the literature. While heart blood was collected as part of the routine autopsy examination, this investigation evaluated the potential for PMR only within the peripheral compartment.

It is also important to note that all specimens were collected prior to evisceration. Therefore, the removal of blood from an unclamped vessel could draw from the central compartment via the inferior vena cava. In this study, we collected two specimens (Specimen 1 and Specimen 3) that were not ligated prior to collection. As previously described, this approach allowed the comparison of blood drug/metabolite concentrations using three commonly employed collection/shipping procedures. Specimens 1 and 3 differed only in their storage and shipping procedures. Therefore, this provided a useful comparison for evaluating analyte stability under these two conditions. Specimen volumes were relatively small (2.0 – 10.0 mL). Nevertheless, the potential contribution of blood drawn from Specimens 1 and 3 via communication with the central compartment may compromise this comparison. Also, we limited the study by only including specimens from decedents who had a relatively short time interval of death and did not include specimens from decedents who had time of death intervals longer than 48 hours based on the investigation of the circumstances of death or who were decomposed. Evaluating drug concentrations after longer postmortem duration offers valuable information, but has been published elsewhere and is beyond the scope of this investigation.

Another significant limitation of this study was that specimens were not split prior to submission for toxicological determination. The ideal statistical design for this study would involve splitting each specimen after collection and submitting one at ambient temperature and the other on dry ice. This would allow for the appropriate statistical comparisons between collection sites isolated as a single variable as well as shipping conditions as a different and potentially co-dependent variable. Instead, we compared three collection/shipping procedures where the collection and shipping method were combined into a single independent variable. We adopted this study design largely due to limited funding to support a large enough sample size

for each drug/metabolite or drug class. With this approach we were able to achieve enough statistical power to detect significant differences between the three specimen collection/shipping procedures for three drugs/metabolites. Additionally, this approach resulted in a large number of different analytes detected.

Difficulty in obtaining informed consent was an unanticipated limitation that was time consuming and resulted in specimens being stored frozen for extended periods. Informed consent protocol required an inordinate amount of time tracking down phone contacts. Often, phones were out of service, calls were never answered, next-of-kin could not be verified, or permission of inclusion into the study was refused. Despite our efforts to insure that no perceived rights had been ignored, the insistence of obtaining a valid informed consent became a setback in achieving the desired number of cases in this study.

Evaluation of analyte stability was not included as a primary objective for this study. Data on drug stability are widely published and include various experiments to define drug/metabolite stability in a number of contexts. The specific statistical design of this study did not allow for characterization of analyte stability, but rather provided a means of comparing concentrations in the three peripheral specimens. Analyte instability is only one potential explanation for the concentration differences detected in the three specimens.

A modification of the original grant submission involved eliminating the analysis of the effect that massaging the leg to obtain specimen may have impacted the results. Objective measurement of the degree of massaging/milking the leg was difficult to establish.

6. IMPLEMENTATION OF POLICY AND PRACTICE

Deaths investigated by the medical examiner and coroner offices across the United States impact public health, public safety and the criminal justice systems. Autopsy information is used by both prosecutors and defense attorneys in civil and criminal trials. Drug poisoning deaths involving prescription medication can lead to litigation against the prescribing physician. Drug poisoning deaths involving illicit drugs can lead to criminal charges against the supplier of the drug. Inaccurate determinations of the cause and manner of death in drug-related fatalities can greatly impact legal proceedings and can have potential adverse consequences for families of the decedents. On-going research in forensic toxicology continually demonstrates that many variables can influence a postmortem specimen drug concentration. Interpretation of postmortem drug concentrations in determining the cause and manner of death, therefore, needs to follow a team approach with consultation between the forensic pathologist, coroner or medical examiner and the toxicologist. This type of team approach helps insure that death certification in possible drug-related fatalities is as accurate as possible based on current scientific knowledge.

In general, comparison of drug concentrations between femoral and iliac venous specimens showed no statistically significant differences. Rigid attention to which peripheral site (iliac or femoral vein) is used to collect the specimen and the way the peripheral blood sample from the iliac and/or femoral veins is performed by isolating and clamping the chosen vein does not appear to be necessary. Many prior research studies investigating PMR of drugs are retrospective and compare drug levels in centrally located blood with levels in peripherally located blood and/or in various tissues. Often these retrospective studies do not or cannot describe how the peripheral blood was obtained. The results of our study may lend credence to those prior research studies looking at drug concentrations in that the careful selection of

peripheral blood from the iliac or femoral vein does not appear to be a significant variable. Concern over the environmental conditions during overnight shipping negatively impacting the stability of the specimen also does not appear to be warranted. Transportation costs can place a significant burden on a coroner or medical examiner's office given the usual chronic state of lack of adequate funding most offices have to face. Offices that must ship toxicology specimens to an outside laboratory are looking for the cheapest alternative to transport their specimens. Offices relying on a commercial carrier such as UPS or FedEx can ship specimens overnight, thereby reducing the amount of time the specimens are exposed to possible adverse conditions like extremes in weather conditions. If significant degradation of the specimens occurred even with overnight delivery, precautions to preserve the specimens, which may include freezing the specimens and delivering them on dry ice to keep them frozen, would need to be taken. The weight of dry ice increases the cost of each shipment considerably. For example, a typical 10-20 specimen package sent overnight with Fed Ex at room temp (no dry ice) costs $14.00. A typical 10-20 specimen package with dry ice costs $37.56. The conclusions from the study indicate that freezing specimens prior to shipment and shipping specimens on dry ice does not provide any advantage over shipping specimens in ambient conditions. Ironically, for certain analytes, freezing of the specimen and shipping on dry ice may actually adversely affect the concentration of the analyte.

Analysis of the data highlighted significant findings that potentially impact interpretation of postmortem drug levels. Large fluctuations in concentrations for some drugs occurred, sometimes greater than a factor of 2, when comparing the peripheral sites indicating that PMR or degradation can occur in these peripheral compartments. More concerning, one or more peripheral specimens were negative for any drug compared to the other specimen(s). A false

negative result was noted for certain drugs in specimens derived from the inguinal region and iliac vein, which were frozen, shipped on dry ice and held in storage for a varying amount of time. False negative toxicologic results will, in most cases, dramatically impact the certification of death.

These findings may influence development of standard operating procedures governing the acquisition of postmortem toxicology specimens during the performance of forensic autopsies. The number of specimens obtained during the routine autopsy should not be confined to only one site. Rather, multiple samples should be obtained from different sites to include peripheral and central compartment, and different specimen types should be collected including urine, tissue such as liver, and possibly vitreous. The acquisition of peripheral blood using the "blind" stick of the femoral vein in the inguinal region seems to be a valid and preferred specimen in addition to these other samples.

This study, along with prior research, emphasizes the need to consider postmortem drug levels in combination with circumstances of death, review of the decedent's drug use pattern and medical history, and scene investigation. Although the general trends in drug concentrations did not differ significantly, radical fluctuations in some drug concentrations and false-negative results for one of more specimens in a set in specific cases underscores the variability that can occur even in controlled conditions that may result in erroneous conclusions. Pathologist and toxicologists must exercise caution when interpreting peripheral blood concentrations and refrain from trying to make diagnoses based solely on the drug level.

7. IMPLICATION FOR FURTHER RESEARCH

Interpretation of postmortem blood drug concentrations can be difficult, owing to many factors, including PMR and drug stability in postmortem specimens. Pre-analytical conditions such as storage of blood and tissue specimens, time interval of death prior to acquisition of specimens, and acquisition of blood from a central or peripheral location among other factors can dramatically alter the interpretation of toxicologic analysis of specimens obtained from deceased individuals. Interpretation of postmortem toxicologic analyses can be influenced by inability to detect drugs, for example, designer drugs for which there is no standard for comparison and by individual differences in metabolism of certain drugs. Thus, drug concentrations must be interpreted in light of results from a complete autopsy, evaluation of drug usage including dosage, duration of drug administration, presence of co-morbidities like heart disease, and consideration of potential drug interactions if more than one drug is present. Problems related to genetic-related differences in metabolism of specific drugs and drug receptor problems on a cellular basis have confounded interpretation of drug levels in the current age, and further studies are needed to address these particular issues.

This prospective study focused on controlled pre-analytical conditions affecting drug concentrations in autopsy specimens. The collection period spanned the time between January 1, 2011 and February 25, 2013. An interesting finding from this study that may warrant further investigation is the apparent loss of or inability to detect a small subset of drugs in the blood samples that were frozen and shipped on dry ice. It is not known whether this phenomenon was due to instability created by the freezing-thawing cycle, whether there was alteration in the chemical structure due to freezing, some other change in the matrix interfering with drug detection, a combination of these possibilities or other reasons not mentioned.

8. DISSEMINATION OF INFORMATION

These findings will be presented as either poster or platform presentations at the American Academy of Forensic Sciences (AAFS). Accepted presentations at this conference will be submitted as a manuscript(s) to AAFS' associated journal, The Journal of Forensic Sciences. Further manscript(s) discussing other aspects of this research not presented at AAFS may be submitted to other appropriate toxicology journals.

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