

# Introduction to Postmortem Studies in Forensic Pathology

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## Objectives

- Compare and contrast a forensic autopsy versus a medical autopsy.
- Describe different types of evidentiary specimen collection in forensic medicine as well as the importance of maintaining chain of custody from person to person.
- Describe types of postmortem specimens collected and used for forensic toxicology as well as other laboratory studies including but not limited to chemistries, genetic studies, microbiological studies, etc.

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## Objectives (continued)

- Describe various analytical methods used to detect drugs and ethanol in forensic toxicology.
- Describe the challenges and pitfalls that exist in the interpretation of postmortem forensic toxicology and know what types of factors may influence laboratory results including but not limited to decomposition, postmortem redistribution, and embalming fluids.
- Describe which types of drugs are most subject to postmortem redistribution, optimal sites for obtaining postmortem blood for toxicological analysis as well as the preferred type of collection tube.

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## Definition of autopsy

- An autopsy (Gr. autopsia for “seen by oneself”) is “inspection of a dead body which has been opened so as to expose important organs either to ascertain the cause of death, or if this is known, the exact nature and extent of the lesions of the disease, and any other abnormalities present”

Webster’s New International Dictionary, unabridged 2<sup>nd</sup> edition, 1956.

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## Objectives of the autopsy per College of American Pathologists

- The evaluation of clinical diagnoses
- The detection and diagnosis of unsuspected diseases
- The study of the cause, nature and development of disease
- Determination of the cause of death
- Assisting in the assessment of the validity, value and appropriateness of diagnostic and therapeutic procedures
- Provision of information to family, physicians, and society
- Quality assurance in medicine
- Medical education and training

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## Forensic versus medical autopsy

- Some differences in a forensic autopsy compared to a medical autopsy:
  - A forensic autopsy does not require permission from next of kin and is ordered by the medical examiner’s office and/or the district attorney general
  - In addition to the previous stated purposes of the autopsy, **the forensic autopsy is performed for legal purposes** with emphasis on documentation of injuries, toxicological findings and collection of evidence
  - The forensic autopsy in TN is a public record; whereas, medical autopsies are confidential.
  - A medical autopsy requires permission from the next-of-kin

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## Medicolegal systems in US

- Varies from state to state
- Coroner versus medical examiner (ME) system
- ME systems vary from state to state
- Who can be a medical examiner in TN
  - Any licensed physician can be a county medical examiner
  - Role of forensic pathologist

## Duty of medical examiner or coroner

- Forensic pathologist (FP) may be the county ME or a consultant to a county ME who is not an FP.
- To determine the cause and manner of death when the death falls under the jurisdiction of the medical examiner or coroner as mandated by the laws in each state
- In general, death requiring medicolegal investigation includes but may not be limited to:
  - Violent deaths (homicide or suspected homicides, suicides, accidents)
  - Suspicious deaths
  - Sudden and unexpected deaths
  - Child deaths

## Sequence of events in a forensic case

- Death investigation by death investigator, medical examiner, coroner, and/or police in some jurisdictions;
  - Identification (tag body), notification of family if a forensic autopsy is going to be ordered by the medical examiner or death investigator or attorney general (permission of the family is NOT required for a forensic autopsy in TN)
  - History with appropriate medical records, scene examination, photography
    - If crime scene, police will process the scene
    - May collect medication bottles from scene if applicable
  - Protect evidence from loss that may be on the body (i.e., bag hands with paper bags, not plastic, semen on body, saliva from bite marks, etc) as well as protect body from being contaminated
  - Transport of body

## Sequence of autopsy continued

- On arrival at forensic center, log the body in and assign a unique autopsy number or identifier
- X-rays if indicated
- External examination: clothed and unclothed
  - Photography
  - Diagrams documenting evidence of injury and natural disease
  - Collection of evidence as indicated (i.e, rape kit, fingernail scrapings, gunshot residue, saliva from bite marks, semen, trace evidence on the body or clothing such as hairs, paint from a hit and run, etc)

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## Sequence of autopsy continued

- Internal examination
  - Head, neck, chest, abdomen or any part of the body deemed necessary
  - Track wounds and collect evidence such as bullets or other material that may have entered and remained in the body
  - Photograph of evidence as well as internal injuries or findings as necessary
- Laboratory examination
- Histologic examination
- All autopsy evidence, including toxicology, requires CHAIN OF CUSTODY documentation which may need to be verified in court
- Finalize autopsy report (time period usually about 2-3 months, sometimes longer depending upon complexity of case)

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## What is Chain of Custody (also known as chain of evidence)

- Proof that any evidence collected during the autopsy (or scene and police investigation) is actually the same evidence ultimately submitted in court
  - Evidence is packaged (evidence tape frequently used to show it has not been tampered with) and appropriate identifiers, the date, the time, and description of the evidence are placed on the packaging
  - To prove the above, must show a “chain” of signatures of persons having contact with the evidence, starting with the person who collected and then handed it off to the next person and then each person the evidence goes through until it reaches the place of final testing or storage of the evidence

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### Recovery of evidence at autopsy

- Recovering fingerprints from skin is sometimes tried in deceased individuals in homicides with close contact (especially rape/homicides)
- Ask your crime lab how they would best like the evidence collected and packaged.
  - Nail scraping versus nail clippings:
  - Fibers from the body, clothes or other trace evidence (i.e. paint from car in hit and run)
- Blood/semen from decedents clothes that could be the assailants
- Saliva from possible bite marks (swab the skin at collect possible bite marks)
  - Forensic dentist to examine bite mark

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### Sexual assault exams

- Rape kit:
  - Combing pubic hair (packaged with comb)
  - Plucking pubic hair (packaged separate from combed hairs)
  - Vaginal, rectal and/or oral swabs
    - DNA, acid phosphatase and/or prostate specific antigen
  - Smear on slide for microscopic (looking for sperm)
  - Bite marks
  - Clothing as evidence
- In living, need to worry about STDs and pregnancy

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### Collection of DNA samples for identification purposes

- Useful in
  - Identification of some deceased individuals
  - Mass disasters
  - Criminal investigations; identification of suspects (i.e. rape, etc)
  - Identification of relatives, especially parents
- Avoid cross contamination (need proper collection, packaging, storage)
- Need samples with nucleated cells
  - At autopsy, collect blood (purple top, blood spot on paper) most of the time; can also use hair WITH follicle, bone, teeth, organs if necessary
  - DNA evidence in criminal cases may be found in semen in rape cases, perpetrator's skin under victim's nails, etc.
  - Bone, teeth for DNA may be used if no blood or acceptable soft tissue
- Mitochondrial DNA; maternally inherited (would not be unique to that individual only)

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### Toxicological Screening Methods (one example from one lab)

- Screening of blood and urine for drugs
  - Specimens extracted by liquid-liquid extraction for acidic and basic drugs, concentrated and then analyzed by thin-layer chromatography (TLC)
  - Colorimetric techniques to screen for some substances
  - Screening for drugs of abuse with enzyme multiplied immunoassay techniques (EMIT)
    - Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and metabolite, opiates, methadone, fentanyl and buprenorphine
- GC-MS (gas chromatography –mass spectrometry) confirmation and quantitation of substances detected with screening procedures

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### Toxicological Screening Methods (one example from one lab)

- Alcohol and other volatiles are screened, confirmed and quantitated by GC-FID (gas chromatography-flame ionization detector) analysis in any biological fluid
  - Detects and quantitates ethanol, methanol, acetone and isopropanol
  - Same technology with different columns and condition to assay ethylene glycol

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### Post mortem specimens for toxicology

- Blood
- Vitreous
- Cerebrospinal fluid
- Urine (may show drugs in urine but not blood)
- Bile
- Fecal material (meconium in babies)
- Stomach contents
- Organs, muscles
- Hair, Nails
- Maggots
- **CHAIN OF CUSTODY VERY IMPORTANT IN DISPOSITION OF ALL EVIDENCE, including toxicology, IN FORENSIC AUTOPSIES**

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## Urine

- Interpretation of positive urine drug screens
  - False positive results may occur on screens
  - Even a true positive urine does not mean that detectable levels of drug are still in the blood
- At autopsy, also useful for detection of glucose, ketones, specific gravity via urine dipstick

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## Vitreous

- Electrolytes (sodium and CL fairly reliable) K increase after death
- Glucose: normally very low or zero; if elevated could indicate diabetes
- BUN, Cr: fairly reliable postmortem
- Ethanol in the vitreous reflects the blood ethanol level several hours prior to death at equilibrium (protected somewhat from postmortem bacterial production of ethanol more than blood)
- May be able to detect other substances such as drugs but can't correlate to blood

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## Stomach contents

- Oral ingestion of intentional overdose of drugs could result in large "clump" of partially digested pill material but not always
  - Frequently don't see pill particles in accidental "overdose"
- If stomach contents are positive on a drug screen, could have diffused from blood into stomach with breakdown of tissues
- Contents of stomach with drugs and especially ethanol may diffuse into adjacent organs and blood, especially hemothorax and especially if stomach is perforated; either postmortem or due to trauma
- After organ donation, blood pools in body cavities and urine may leak out of bladder and admix with blood in cavities when kidneys have been taken; could perfuse into stomach (Use blood obtained at organ donation for tox)
- A blind heart stick for tox could penetrate into esophagus and regurgitated stomach contents could contaminate the specimen

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## Factors affecting postmortem (PM) toxicology

- Decomposition (increased ethanol due to bacterial production), chemicals produced during decomposition may interfere with detection of drugs, degradation of drugs over time
- Postmortem (PM) redistribution (amitriptyline and other basic drugs)
- Levels of drugs do not necessarily correlate with whether or not they caused death. (tolerance to drugs as well as normal levels in combination being synergistic and causing death)
- Embalming fluids

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## Postmortem redistribution and effects on drug levels

- Some drugs with high volumes of distribution diffuse out of tissues (particularly the lungs, liver and heart) after death giving elevated postmortem blood levels
- More likely with basic, lipophilic drugs
- Examples of drugs demonstrating this phenomenon are amitriptyline and other tricyclic antidepressants, amphetamines, etc.
- Draw postmortem blood from less central locations such as the femoral vessels in order to decrease the effects of postmortem redistribution

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## Preferred sites of blood collection postmortem in descending order

- Femoral vessels (preferred site)
- Subclavian vessels
- Root of aorta
- Pulmonary artery
- Superior vena cava
- Heart
- Least preferred would be blood in body cavities

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## Containers for blood postmortem

- Use glass containers, not plastic (polymers in plastics could leach out and interfere with drug analysis, masking some compounds or volatile toxins could be absorbed by the plastic and not detected on tox)
- Types of tubes collected include:
  - EDTA tube (DNA sample)
  - Gray top tubes with potassium oxalate (anticoagulant) and **sodium fluoride** as a preservative preventing enzymatic activity best for toxicology
- If unsure what type of specimen collection technique or container to use, TALK TO YOUR TOX LAB

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## Decomposition effects on drug levels and detection

- Drug concentrations may change due to:
  - Bacteria
  - Interference in detection due to decomposition chemicals
  - Continued metabolic activity of some tissues after death
  - Postmortem redistribution
- **Embalming fluids**
  - Remaining blood diluted
  - Chemical interference with detection

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## lab methods for ethanol detection

- Chemical tests (if ethanol or ANY other volatile reducing substance added to K dichromate and sulfuric acid, chromate converts to the chromic ion causing a color change from yellow to green)
- Enzymatic tests (Ethanol is converted to acetaldehyde by alcohol dehydrogenase with NAD as a cofactor, NADH is produced and measured which quantitates the ethanol)
- Gas Chromatography (**used in detecting other volatiles as well, such as methanol, acetone and isopropanol**)

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## Ethanol

- Blood alcohol concentrations used to define legal intoxication are given in WHOLE blood concentrations, not plasma or serum; but some hospital lab measurements may be from using serum or plasma, NOT whole blood.
  - The serum ethanol will be higher than whole blood ethanol since a volume of serum will have 12-20% more water than an equal volume of whole blood
  - Serum ethanol to whole blood ethanol ratio averages 1.18 (ranges from 1.10 to 1.35)

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## Specimens for ethanol analysis at autopsy

- Blood [central collection could possibly be contaminated by postmortem diffusion of ethanol through the stomach (or rupture of stomach) into surrounding tissue and blood vessels and into pleural cavity]
- Vitreous (1.2 times the blood ethanol at equilibrium) Reflects the blood ethanol level 1-2 hours prior to death
  - The only time vitreous ethanol is lower than blood ethanol is during the absorptive phase
- Urine (1.3 time blood ethanol if collected in the ureter)
- Muscle (thigh) isolated from other organs and resistant to decomposition with blood to muscle ration of 0.094 +/-0.086

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## Factors affecting ethanol levels

- Evaporation from the specimen collected may occur if collected in a container if a large air space exists between the lid and the specimen
- Postmortem production of ethanol in the body can occur due to bacterial fermentation of glucose to ethanol
  - vitreous and urine ethanol will usually be negative if blood ethanol is from bacterial production
- Gray top tube with NaF as preservative prevents ethanol production after specimen collected
- Embalming fluid generally does not contain ethanol but blood will be diluted

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## Cocaine

- Cocaine stability in postmortem blood:
  - Fluoride in gray top tube inhibits further enzymatic hydrolysis of cocaine to ecgonine methyl ester
  - However, fluoride may prevent enzymatic hydrolysis, it does not prevent spontaneous hydrolysis of cocaine to benzoylecgonine
    - “Even after the addition of 2% sodium fluoride, a 25% decrease in cocaine concentrations was observed at room and refrigerated temperatures within 5 and 80 days, respectively.” Barry Levine. Principles of Forensic Toxicology, Second Edition.
    - Another study showed that acidifying the blood to pH5 inhibited nonenzymatic hydrolysis
- Benzoylecgonine and ecgonine methyl ester may also undergo spontaneous hydrolysis postmortem which is also pH and temperature dependent

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## Interpreting blood levels of cocaine

- “therapeutic” levels of cocaine can still cause acute toxicity and death
- In addition to acute cocaine toxicity, consider chronic effects as well, i.e., myocardial hypertrophy and fibrosis and increase risk for arrhythmias
- “lethal” levels of cocaine may sometimes be detected in individuals who clearly had another cause of death
  - Cocaine is also prone to postmortem redistribution
- Excited delirium: cocaine-induced psychosis characterized by severe hyperthermia, extreme agitation and delirium, and sudden death; no clear cut correlation with levels

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## Opiates/Benzodiazepines

- May be detected in the urine drug screen but not the blood drug screen (possibly below detection limits on screen); However, when blood quantitations are obtained, may have significant measurable amounts present.
  - Some opiates such as fentanyl may not be detected
  - Clonazepam may not be detected on benzo screen.
  - Zolpidem (not opiate or benzo) usually not detected on drug screens.
- Heroin (synthesized by Bayer in 1898 by the acetylation of the 2 hydroxyl groups of morphine, in the body is deacetylated to 6-acetyl morphine and then morphine)
  - Heroin not usually detected due to rapid metabolism (minutes) to 6-acetyl morphine (half life of 0.6 hours) and then morphine
  - **May only detect morphine in a heroin OD, need to at least detect 6-acetyl morphine to call heroin OD**

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## CNS depressant drugs combined with ethanol

- Ethanol may greatly potentiate the effects of opiates/benzodiazepine or other CNS depressant drugs resulting in toxicity or death
  - high levels of ethanol will compete with other CYP2E1(part of the cytochrome p450 family of enzymes) substrates and delay drug catabolism, potentiating the CNS depressant effects of drugs such as opiates, benzodiazepines, etc.
  - Ethanol binds to the gamma-aminobutyric acid (GABA) receptor in the CNS (so do benzos as do various sedative/hypnotics)

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## Synergistic effects of multiple CNS depressant drugs

- CNS depressant drugs used in combination may have serious toxicity sometimes resulting in death even at “therapeutic” ranges
- Mechanism of death: CNS and respiratory depression
- Conversely, an individual may be tolerant to a toxic or lethal drug level with chronic use

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## Marijuana

- Made from the Cannabis sativa plant which contains the psychoactive substance delta-9-tetrahydrocannabinol (THC)
- Detection of cannabinoid on urine drug screen may not indicate recent marijuana usage.
- If important to know if under influence acutely, will need quantitation and interpretation of the levels of various cannabinoids in the blood

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### Other studies obtained on posmortem blood

- Hemoglobin A1C on purple top
- Serum hormone levels
- Genetic studies
- Not good for Blood glucose, CBC, Coagulation studies, enzymes, electrolytes
- Overall poor quality of postmortem specimen and hemolyzed serum may interfere with various analyses

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