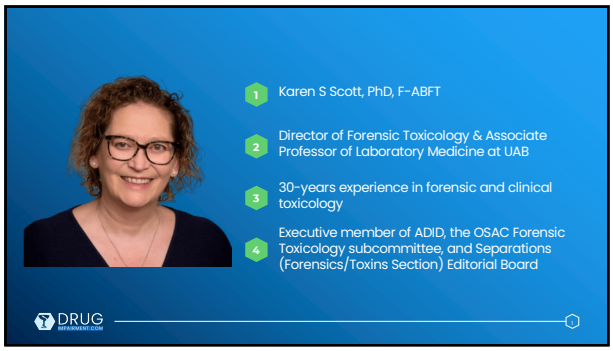
Karen S Scott, PhD, F-ABFT
Director of Forensic Toxicology & Associate Professor of Laboratory Medicine at UAB
30-years experience in forensic and clinical toxicology
Executive member of ADID, the OSAC Forensic Toxicology subcommittee, and Separations (Forensics/Toxins Section) Editorial Board
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1


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Overview of Matrices in Forensic Toxicology Part 1
Karen S. Scott, PhD, F-ABFT

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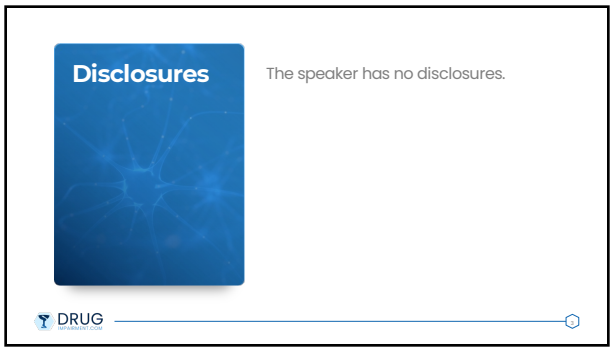
Disclosures
The speaker has no disclosures.
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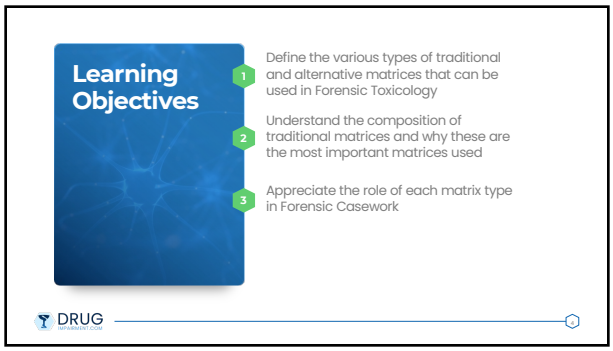
Learning Objectives
1
Define the various types of traditional and alternative matrices that can be used in Forensic Toxicology
2
Understand the composition of traditional matrices and why these are the most important matrices used
3
Appreciate the role of each matrix type in Forensic Casework
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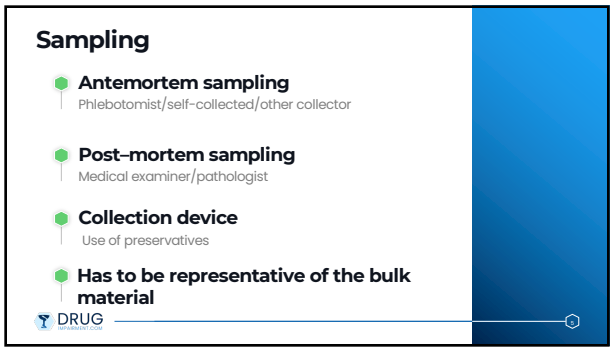
4


Sampling
Antemortem sampling
Phlebotomist/self-collected/other collector
Post-mortem sampling
Medical examiner/pathologist
Collection device
Use of preservatives
Has to be representative of the bulk material
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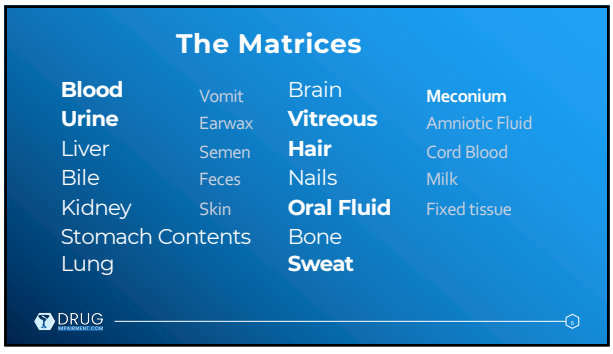
5


The Matrices
Blood
Urine
Liver
Bile
Kidney
Stomach Contents
Lung
Vomit
Earwax
Semen
Feces
Skin
Brain
Vitreous
Hair
Nails
Oral Fluid
Bone
Sweat
Meconium
Amniotic Fluid
Cord Blood
Milk
Fixed tissue
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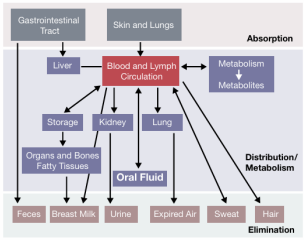
6

Inter-relatedness of ADME & Matrices



https://documents.thermofisher.com/TFS-Assets/CMD/brochures/br-65157-drug-traces-sample-matrices-ebook-br65157-en.pdf

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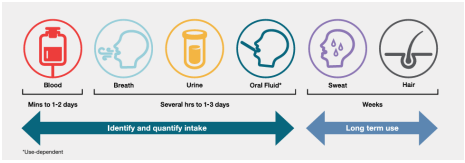
How long do drugs stay in matrices?



https://documents.thermofisher.com/TFS-Assets/CMD/brochures/br-65157-drug-traces-sample-matrices-ebook-br65157-en.pdf

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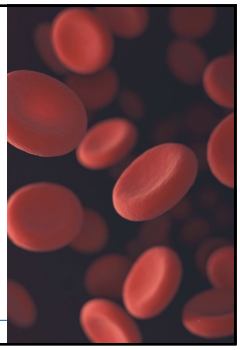
What is Blood?

- A transport fluid that is pumped by the heart to all parts of the body
- Transports O_2 & nutrients to cells and carries away $CO_2$and waste products
- Also a tissue as it is a collection of specialized cells that serve particular functions
- These cells are suspended in plasma which makes blood a fluid
- Flow is required for life – consistency would become unfavorable to maintain a suitable balance and death would occur
  - Lungs – oxygenation
  - Kidneys – water balance & waste removal
  - Endocrine system – hormone transport
  - GI tract - nutrients



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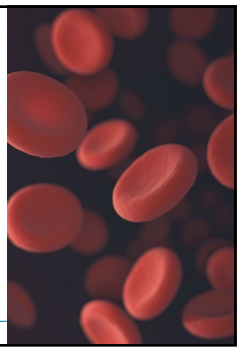
Blood Composition

- **Plasma:** The liquid portion, making up about 55% of blood, is mostly water (around 92%) and contains proteins, glucose, mineral ions, hormones, and waste products
- **Red Blood Cells (RBCs):** These carry O_2 from the lungs to body tissues and transport CO_2 back to the lungs. Contain hemoglobin, a protein that binds O_2 and gives blood its color
- **White Blood Cells (WBCs):** These are part of the immune system, helping the body fight infections and parasites
- **Platelets:** Small cell fragments that play a crucial role in blood clotting to prevent excessive bleeding after injury



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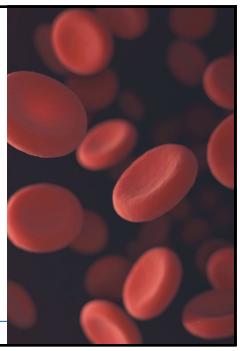
Additional blood facts

- Arteries carry O_2-rich blood from the heart to tissues, while veins return O_2-poor blood to the heart
- Blood cells are produced in the bone marrow from stem cells
- RBC production is regulated by erythropoietin (EPO), a hormone primarily produced by the kidneys
- Blood accounts for ~7–8% of body weight, with an average adult having ~5–6 L
- Blood pH ranges from pH 7.35 to 7.45
- Its temperature is slightly higher than normal body temperature, around 38°C (100.4F)



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Why Test Blood?

1. Often regarded as the gold standard in forensic toxicology as it provides a real-time snapshot of the substances present in the body at the moment of collection
   - Makes it most reliable for establishing the state of intoxication or poisoning at a specific time
2. Can reflect the current concentration of drugs and toxins in circulation
   - Critical in cases such as impaired driving, where the time of intoxication needs to be established
3. As blood contains the same compounds that affect the brain and other organs, its composition directly reflects the immediate physiological impact of substances.
   - This makes it invaluable for determining impairment levels and for postmortem toxicology
4. Decades of research, regulatory frameworks, and quality control protocols
   - Makes it the most trusted and widely accepted matrix for legal cases

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Why Not Test Blood?

- Invasive matrix which requires trained personnel for collection
- Some drugs have a very short residence time in blood
- Other matrices such as urine, vitreous or oral fluid may be more appropriate to collect in some circumstances
- These are useful when non-invasive collection is needed or in post-mortem cases
- However, these have different detection windows and may not reflect the time of exposure

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What is Urine?

- A liquid waste produced by the kidneys to remove excess water, salts and metabolic byproducts from the blood
- Primary purpose is to maintain the body's chemical balance, regulate water levels and eliminate toxins that the body cannot use
- Healthy human urine is mostly water, making up 91% to 96% of its volume. The remaining 4% to 9% consists of dissolved waste substances and minerals, including:
  - **Urea**: a nitrogen-rich compound formed when the liver breaks down proteins
  - **Uric acid**: a byproduct of purine
  - **Creatinine**: a waste product from muscle metabolism
  - **Electrolytes**: e.g. sodium, potassium, and phosphorus
  - **Pigments**: like urochrome (responsible for yellow color)

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Why Test Urine?

1. Offers non-invasive collection, high analyte concentrations, and a broad detection window for many drugs and their metabolites
2. Often contains higher concentrations of drugs and their metabolites than blood, making it easier to detect substances even at low doses
3. Many substances remain detectable in urine for days, weeks, or even months after use, depending on the drug and usage pattern
4. Can detect both the parent drug and its metabolites
5. Can reveal patterns of drug use that blood tests alone cannot, such as repeated or chronic exposure, even when blood results are negative

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Why Not Test Urine?

- While urine is valuable, it cannot always determine the exact timing or quantity of drug use
- Urine is vulnerable to adulteration or tampering
- Interpretation must be done in the context of other evidence and, when possible, compared with blood or other matrices
- Considerations for phase II metabolites and additional sample preparation

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Samples for Case Types

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Workplace Drug Testing

Blood: recommended for reasonable suspicion or post-incident testing.
Oral Fluid: recommended for pre-employment, random, reasonable suspicion and post-incident testing
Urine: recommended for pre-employment and random testing.
Hair: may be employed for pre-employment testing and random testing in a deterrence program

Mandatory guidelines exist for urine and oral fluid

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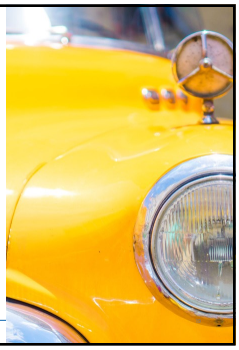
18

Human Performance – DUI/DUID

- Blood
- Oral fluid
- (Urine)
- Hair for driving license reinstatement in repeat offenders



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Human Performance – DFC

- Urine (up to 5 days)
- Blood (up to 48 hours)
- Head hair
  - Sample 1: 4-6 weeks after
  - Sample 2: Taken if 1st sample is positive
  - Advise complainant not to treat hair between collections
  - Hair **must** be segmented
- Vomit

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Poisoning - Living

Acute
- Blood
- Urine
- Vomit (gastric lavage)

Chronic
- Blood
- Urine
- Hair
- Nails

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Postmortem

- Peripheral Blood
- Central Blood
- Other blood including any antemortem samples
- Vitreous
- Urine
- Bile
- Tissues
- Stomach contents
- Maggots
- Hair
- Bone
- .....

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22

Abstention

- Urine
- Hair
- Sweat
- Blood

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23

Next Up..

Describe 5 different alternative matrices

Look at stability and storage of these alternative matrices

Discuss how the alternative matrices are prepared for analysis

Finish off these courses by discussing some case examples

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24


CONTACT ME
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karenscott@uabmc.edu
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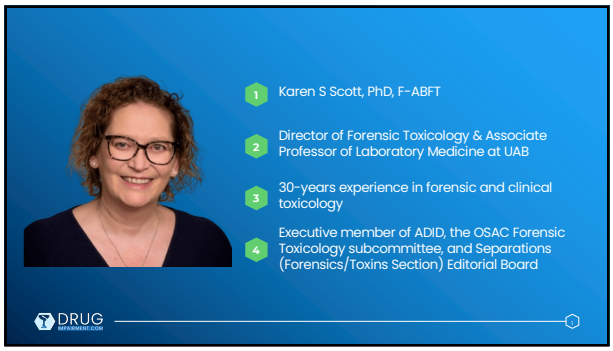
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1


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Overview of Matrices in Forensic Toxicology Part 2
Karen S. Scott, PhD, F-ABFT

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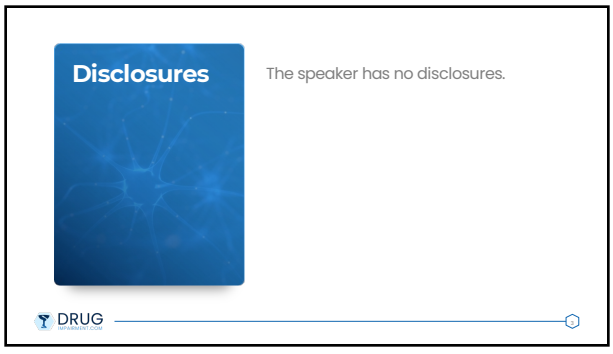
Disclosures
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Learning Objectives

Vitreous
Hair
Oral Fluid
Sweat
Meconium

1. Describe the constituents of each matrix
2. Discuss how drugs are incorporated into each matrix
3. Summarize the sampling, collection and storage considerations for each matrix in forensic toxicology

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What is Vitreous?

- **Clear, gel-like substance that fills the space between the lens and the retina**
  Plays a crucial roles in maintaining eye shape and supporting vision
- **Occupies ~ 80% of eyes volume**
  Composed of 99% water & 1% collagen, glycosaminoglycans, electrolytes and proteins. Also contains phagocytes to keep eye clean
- **Functions:**
  Maintains eye shape & structural integrity; Light transmission; Shock absorption; & nutrient distribution
- **Also has metabolic functions**
  Oxygen transport, glucose and lactic acid storage

www.imaios.com/en/e-anatomy

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How do drugs get there?

- Blood concentration, membrane permeability, lipid solubility, protein binding, size, pK_a, and integrity of the blood retinal barrier all affect transport
  - Across blood retinal barrier - main
  - Across ciliary body and aqueous humor
- Mainly by passive diffusion from higher concentrations in blood
- Osmotic pressure, convection, active transport and fluid motion also contribute to drugs in VH
- Postmortem drugs may continue redistributing as the membranes break down

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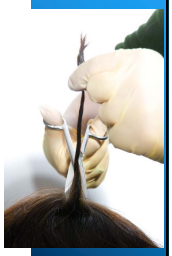
What is Hair?

- **Keratinous substance that covers surface of most mammals**
  Rich in cysteine which forms strong disulfide bonds giving hair its rigidity and resilience
- **Contains 12-15% water**
  Maintains flexibility and softness, prevent brittleness and contribute to health
- **Lipids**
  Inside and on surface provide lubrication and protect against moisture loss
- **Melanin**
  1-3% by weight in natural colored hair. Eumelanin & pheomelanin.
- **Structure**
  Hair follicle (below skin) and hair shaft. (visible part)



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How do drugs get there?

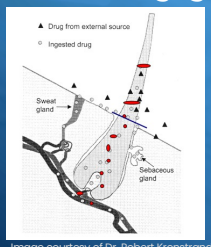

Image courtesy of Dr. Robert Kronstrand

- Primary route is via blood stream through the dermal papilla during anagen phase
  - Affected by lipophilicity, polarity, pK_A
  - Melanin affinity (hair color)
- Sweat and sebaceous gland deposition
  - More important for lipophilic drugs that accumulate in skin reservoirs
- External deposition
  - Drugs in smoke, dust, powders, contaminated surfaces (hand to head)
  - Important in child custody cases
  - Decontamination procedures are important as is use of metabolites
- Once inside drugs are trapped in a charged state

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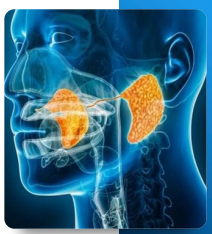
What is Oral Fluid?

- **Primary component is saliva**
  - Viscous liquid with pH 5.8-7.4
  - 94-99% water
  - Proteins/glycoproteins
  - Organics (hormones, vitamins, immunoglobulins, drugs)
  - Inorganics from blood
- **Also contains:**
  - Gingival crevicular fluid
  - Cellular debris
  - Bacteria/microorganisms
  - Food residues
- **Dynamic fluid based on flow of saliva**
  - 500-1500 mL/day



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How do drugs get there?

- From blood stream to salivary glands into saliva
- Directly into mouth from oral administration, smoking, chewing etc.

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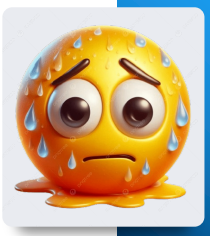
What is Sweat?

- **Watery fluid produced by sweat glands in the skin**
  Regulates body temperature and supports skin health
- **Two types of glands**
  - Eccrine
  - Apocrine
- **Controlled by the hypothalamus**
  - Temperature sensors on skin and body - signal when body temperature rises
  - Acetylcholine is released to trigger sweat production
- **Other factors**
  Spicy food, emotional stress and certain medications can stimulate (or prevent) sweating



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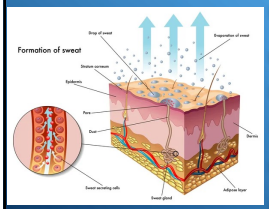
11

How do drugs get there?



- Mainly through passive diffusion from the blood
- Favors small lipophilic drugs
- Active transport of charged and polar drugs
- Lipophilic drugs may partition into sebum produced by sebaceous glands
- Ion-trapping of weakly basic drugs may occur as sweat is slightly acidic (pH 4.5-6.5)
- Drugs in interstitial fluid may move into sweat ducts or onto skin surface during sweating

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What is Meconium?

- **1st stool of a newborn baby**
  Contains materials ingested in-utero
- **Viscous and sticky like tar and almost odorless**
  Dark olive green in color
- **Usually passed within 24-48 hours of birth**
  Consistency changes to feces as milk is ingested
- **Composition**
  Intestinal epithelial cells, lanugo, mucus, bile, water and amniotic fluid

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How do drugs get there?

- Meconium begins developing around 12 to 16 weeks of gestation
- A primary route of drugs entering meconium is through fetal swallowing of amniotic fluid
  - Drugs and metabolites cross placenta, enter fetal circulation, get filtered by fetal kidneys, excreted in urine and released into amniotic fluid
- Drugs also enter through circulation of bile acids. Drugs and metabolites are processed by liver and excreted into the gut

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Vitreous

- **Antemortem sampling**
  N/A
- **Post-mortem sampling**
  Collect all available from both eyes
- **Sample collection**
  Typical collected in a red top tube
- **Sample storage**
  Should be stored in fridge while samples are being tested and frozen once tests completed

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Hair

- **Antemortem sampling**
  Collect pencil thickness from posterior vertex of head close to scalp
- **Post-mortem sampling**
  Collect pencil thickness from posterior vertex of head close to scalp & also a plucked sample
- **Sample collection**
  Collected sample placed into a foil sheet, then secured for transport
- **Sample storage**
  Should be stored at room temperature in dark, dry conditions

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Oral Fluid

- **Antemortem sampling**
  Collected using a OF collection device
- **Post-mortem sampling**
  Can be collected by swabbing mouth
- **Sample collection**
  OF plus buffer in collection device
- **Sample storage**
  Should be stored in fridge while samples are being tested and frozen once tests completed

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Sweat

- **Antemortem sampling**
  Sweat patch placed directly on skin for appropriate amount of time e.g. one week
- **Post-mortem sampling**
  N/A
- **Sample collection**
  Collected directly onto swab
- **Sample storage**
  Should be stored at room temperature in dry conditions

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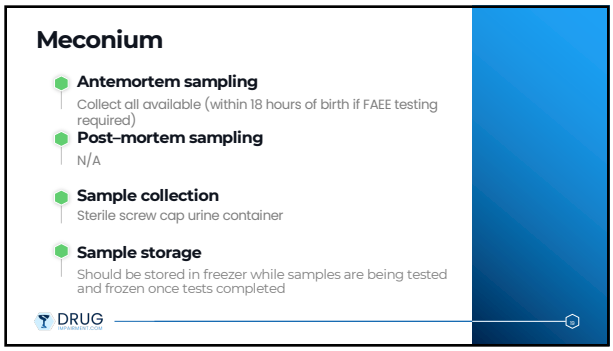
Meconium
Antemortem sampling
Collect all available (within 18 hours of birth if FAEE testing required)
Post-mortem sampling
N/A
Sample collection
Sterile screw cap urine container
Sample storage
Should be stored in freezer while samples are being tested and frozen once tests completed
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Overview of Matrices in Forensic Toxicology Part 3
Karen S. Scott, PhD, F-ABFT

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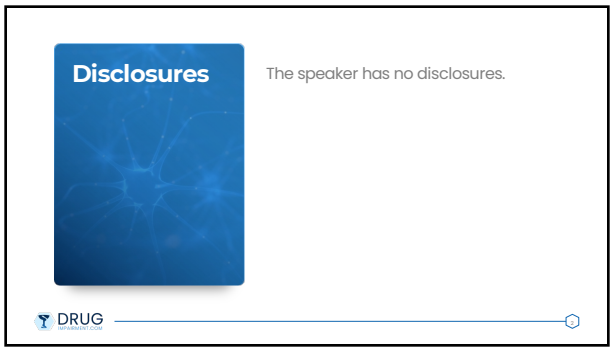
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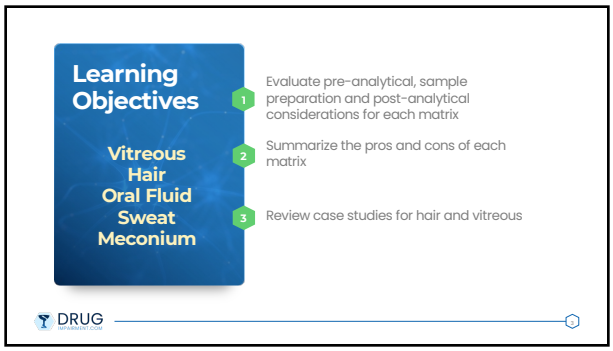
Learning Objectives
Vitreous
Hair
Oral Fluid
Sweat
Meconium
1
Evaluate pre-analytical, sample preparation and post-analytical considerations for each matrix
2
Summarize the pros and cons of each matrix
3
Review case studies for hair and vitreous
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3

Vitreous Preparation & Analysis - Considerations

Pre-analytical
- Sample typically won't have preservatives due to electrolytes testing
- May cause stability issues with some analytes
- Sample protected from PMR so may give good reflection of antemortem concentrations
- Requires distribution to equilibrium
- Limited literature for many drugs
- Sample volume may be limited

Sample Preparation
- Typically, easy to pipette although may be viscous
- Typically, drug methods developed alongside blood methods via matrix matching
- Most commonly tested for vitreous electrolytes and volatiles

Post Analytical
- Useful in forensic toxicology partly because it is relatively isolated and less affected by immediate postmortem redistribution compared with blood
- Metabolic lag compared to blood
- Analytes like ethanol, glucose, and some drugs are sometimes measured there for these reasons

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Hair Preparation & Analysis - Considerations

Pre-analytical
- Drugs may appear in the hair root within 30 min to 1 h depending on melanin content and drug properties
- The incorporated portion won't be detectable above the scalp until 5-10 days after ingestion
- Hair grows around 1cm/month
- Drugs are "permanently" trapped but concentrations can decline due to degradation
- Test hair from posterior vertex as highest % in anagen phase
- If not available test body hair

Table 1
Published growth rates of human head hair.

Year	Reference	Growth Rate (cm/month)		
		Min.	Max.	Avg.
1951	[15]	0.84	1.15	0.98
1964	[38]	0.76	0.96	0.86
1964	[39]			0.94[a]
1992	[40]	0.84	1.37	1.12
1996	[41]	0.65	2.2	1.4[a]
2004	[42]	0.95	1.12	1.04[a]
2005	[43]			1.04
2007	[16]	0.73	1.48	1.11[a]

[a] Average growth rate not reported in reference, so midpoint of range established by the minimum and maximum growth rates used to represent the average growth rate.

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Oral Fluid Preparation & Analysis - Considerations

Pre-analytical
- One of the simplest sample collections
- Sample is collected directly onto an absorbent pad
- Pad is placed into a buffered solution for shipping to lab
- Collection affected by drugs that reduce production of saliva (dry mouth)
- Reduced risk of sample adulteration
- Good short-term stability of drugs in collection devices

Sample Preparation
- Can directly analyze buffered sample but may get matrix effects from food and other oral cavity contaminants
- (if donor has eaten recently, advised to rinse mouth and wait 10 minutes before collection)
- Sample volume may be limited depending on labs testing strategy

Post Analytical
- Good indicator of recent consumption
- Detection of drugs within an hour of use
- Good correlation of OF and blood concentrations for some drugs
- Not good for historical drug use

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Sweat Preparation & Analysis - Considerations

Pre-analytical
- Adhesive patch with absorbent pad applied to clean skin for 7-14 days
- Collects about 2 mL per week
- Sample volume may be limited
- Patch applied and removed under observation
- Patch is waterproof so user can shower etc. as normal
- May be embarrassing for some users

Sample Preparation
- Drugs extracted from patch into suitable solvent for screening or confirmation test
- Sample volume may be limited depending on labs testing strategy

Post Analytical
- Drugs monitored for length of time patch is worn
- Usually sent to a bespoke testing lab with short turnaround times
- Consideration of users' environment when interpreting results
- Consideration of users' past drug use when interpreting results
- Not accepted yet in all jurisdictions

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Meconium Preparation & Analysis - Considerations

Pre-analytical
- May be passed in-utero (post-term pregnancies or fetal distress)
- Non-absorbent liner placed into diaper so that meconium does not adhere to diaper
- Must make sure meconium does not dry out between collections
- For FAEEs sample must be collected within 18 hours and must be refrigerated

Sample Preparation
- Recommended that samples are stored in the freezer prior to analysis
- After thawing, samples are weighed, homogenized in buffer and centrifuged
- Hydrolysis may be required
- LLE and SPE methods exist in literature followed by GC or LC MS

Post Analytical
- Used to determine maternal exposure to drugs, alcohol, tobacco and environmental pollutants
- Gold standard for prenatal drug exposure

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Summary of Pros & Cons

	Pros	Cons
Vitreous	Isolated and less affected by postmortem changes	Post mortem only
Hair	Long term drug history Good for late reporting cases	Not for recent use Complicated preparation Difficult to relate to dose
Oral Fluid	Can mimic blood Non-invasive	Limited volume Contamination from oral cavity
Sweat	Non-invasive Continuous monitoring 7-14 days Tamper-evident patches	Vulnerable to external contamination Drugs already deposited in skin will cause false positive Amount of sweat is not monitored
Meconium	Drugs from 2nd trimester through birth Immediate availability	Difficult to collect and handle Misses 1st trimester exposure Not time specific

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Case Study One – Hair Analysis

Two-year-old child tested positive for 15 drugs

Background

- Some shared custody between mom and dad
- Caregivers denied any prescriptions for relevant drugs
- Mother admitted to smoking methamphetamine
- Father also uses methamphetamine & MDMA
- Other drugs that may have been used in the home included MDMA and cocaine
- Only breastfed on the day he was born
- Positive urine test for methamphetamine started inquiry

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Hair Analysis Results

11 drugs and 4 metabolites

With the exception of BE all are active compounds

How could they get into the child?

Some of the drugs could be smoked – external contamination?
Many are usually taken orally
Parents deny use so could not be from parental sweat?

Concentrations of many of the drugs are relatively low

Exception is methamphetamine – consistent with medium level of use – urine test results indicates ingestion
Ketamine result also relatively high

Drug/Metabolite (detection limit)	Segment 1 Concentration (ng/mg)	Segment 2 Concentration (ng/mg)	Drug or metabolite
Methamphetamine	>5	~4.1	Drug*
Amphetamine	~0.3	~0.4	Metabolite*
MDMA	~0.7	~0.9	Drug*
MDA (0.2 ng/mg)	Not detected*	Not detected*	Metabolite*
Cocaine	~0.7	~0.8	Drug*
Benzoylecgonine	~0.05	~0.05	Metabolite
Ecgonine methyl ester (0.05 ng/mg)	Not detected	Not detected	Metabolite
Codeine	~0.2	~0.3	Drug*
Morphine (0.2 ng/mg)	Not detected*	Not detected*	Metabolite*
Oxycodone	~0.5	~0.5	Drug*
Tramadol	~0.2	~0.2	Drug*
Diazepam	~0.2	~0.2	Drug*
Nordiazepam	~0.03	~0.04	Metabolite*
Lorazepam	~0.02	~0.03	Drug*
Zopiclone	~0.04	~0.04	Drug*
Ketamine	~1.9	~2.2	Drug*
Norketamine	~0.1	~0.1	Metabolite*
Promethazine	~0.5	~0.5	Drug*

* Active drug or metabolite

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Drug	Prescription or Illicit Drug	Most Common Route(s)	Alternative Route
Methamphetamine	Illicit	Smoking	Insufflation*, injection, oral
MDMA	Illicit	Oral	Insufflation, occasionally smoked
Cocaine	Illicit	Smoking (crack), insufflation, injection	-
Codeine	Prescription	Oral	Insufflation, injection, smoking
Oxycodone	Prescription	Oral	Insufflation, injection, smoking
Tramadol	Prescription	Oral	Insufflation, injection, smoking
Diazepam	Prescription	Oral	Insufflation, injection
Lorazepam	Prescription	Oral	Insufflation, injection
Zolpidem	Prescription	Oral	-
Ketamine	Illicit	Smoking, Insufflation, injection	Oral
Promethazine	Prescription	Oral, injection	-

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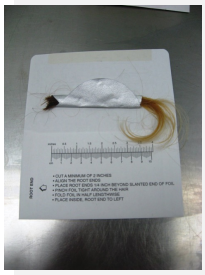
Conclusions

Methamphetamine and ketamine exposure has occurred during time represented by hair

- Methamphetamine is consistent with regular use of the drug
- Cannot rule out ingestion
- Cannot comment of amount of either methamphetamine or ketamine ingested or frequency of ingestion

For MDMA, cocaine, codeine, oxycodone, tramadol, diazepam, lorazepam, zopiclone and promethazine the concentrations and patterns of exposure are more indicative of passive exposure via sweat, smoke or surface contamination



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Case Study Two - Vitreous

Fentanyl child case

- Two-year-old Black Female admitted to ED after being found unresponsive in bed with Mother
- Mother had taken child to bed in the afternoon (1430); found by father on return from work (~1600)
- According to Staff Nurse was given 6 round of Epi before arriving at the ED and was in full arrest when she arrived (1647)
- Pronounced soon after arrival (1656)

- Residence was unkempt and drug paraphernalia was found in the same room as the child

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PM Findings

- The examination did not detect any natural disease or injuries
- Lungs congested and heavy (LL 136 g, RL 107 g)
- Excess fluid in pleural cavities
- Petechiae on heart

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Toxicology Results

Only fentanyl and metabolite detected

- As this was a child, several matrices were collected and tested.
- Included several non-traditional matrices (vitreous and gastric contents)
- One blood sample sent to reference lab for confirmation of metabolites

How could they get into the child?

- The home was stated to be unkempt so possibly child got into parent's drug supply?
- High level in gastric content so likely oral ingestion
- Hair not collected so not possible to evaluate previous exposures from the home environment

Matrix	Drug	Method	Conc. (ng/mL)
Aorta & Pulmonary Trunk Blood	Fentanyl	GC/MS	>75
Pulmonary Trunk Blood	Fentanyl	GC/MS	52
Pulmonary Trunk Blood	Fentanyl	LC/MS/MS	63
Pulmonary Trunk Blood	Norfentanyl	LC/MS/MS	1
Iliofemoral Blood	Fentanyl	GC/MS	19
Bile	Fentanyl	GC/MS	47
Vitreous	**Fentanyl**	**GC/MS**	**10**
Gastric Contents	Fentanyl	GC/MS	757

Central : Peripheral 2.7:1 Parent : Metabolite 63:1

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Conclusions

- **Cause of death: Fentanyl toxicity**
- **Manner of death: Undetermined**
- **Means of death: Drug-opioid**

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