Sample, Sampling, Preserving and Chain of Custody in Toxicological Analysis

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مقدمه

- نمونه برداری صحیح (Sampling) و ارسال درست نمونه به آزمایشگاه نه تنها در آزمایشگاه سم شناسی قانونی (Forensic Toxicology Laboratory) بلکه در تمامی آزمایشگاه ها در رسیدن به هدف اصلی که دستیابی به پاسخی دقیق، صحیح و قابل اعتماد (Precise, Accurate and Reliable) است نقش بسزایی دارد.

- چه بسا در مواردی عدم آگاهی از نحوه برداشت و ارسال نمونه به آزمایشگاه مسئول آزمایشگاه را در ارائه پاسخ و پزشک معاین را در تفسیر نتایج به گمراهی برده است.

- نمونه برداری مهمترین مرحله در آنالیز داروها و سموم محسوب می گردد.

- نمونه برداری در موارد پس از مرگ (Postmortem Sampling) از فردی به فرد دیگر، بسته به شرایط، نوع درخواست پزشک معاین، جنبه های قضایی و در دسترس بودن نمونه متغیر است.

- نمونه برداری شامل انتخاب صحیح نمونه، بسته بندی، برچسب زنی، حفظ و ارسال آن به آزمایشگاه می باشد.

Introduction

- Analytic Toxicology involves application of analytic chemistry for the qualitative or quantitative estimations of chemicals that may exert adverse effects on living organisms.

- Forensic Toxicology is the use of toxicology for purposes of the **law**.

زنجیره حفاظت نمونه و پرونده های ارجاعی به آزمایشگاه

- از منظرعلوم قانونی کلیه مراحل برداشت و ارسال نمونه، انتقال درون بخشی یا خارج بخشی، انجام آزمایشات، معدوم کردن نمونه ها و پاسخدهی به درخواست های آزمایش باید مستند سازی شوند.
- مستند سازی می تواند به شکل کاغذی بوده و تمامی شواهد باید بایگانی شوند.
- چنانچه شواهد و مستندات به شکل الکترونیک تهیه می شوند نیز بایگانی و ثبت کلیه مراحل اجباری و الزامی است.

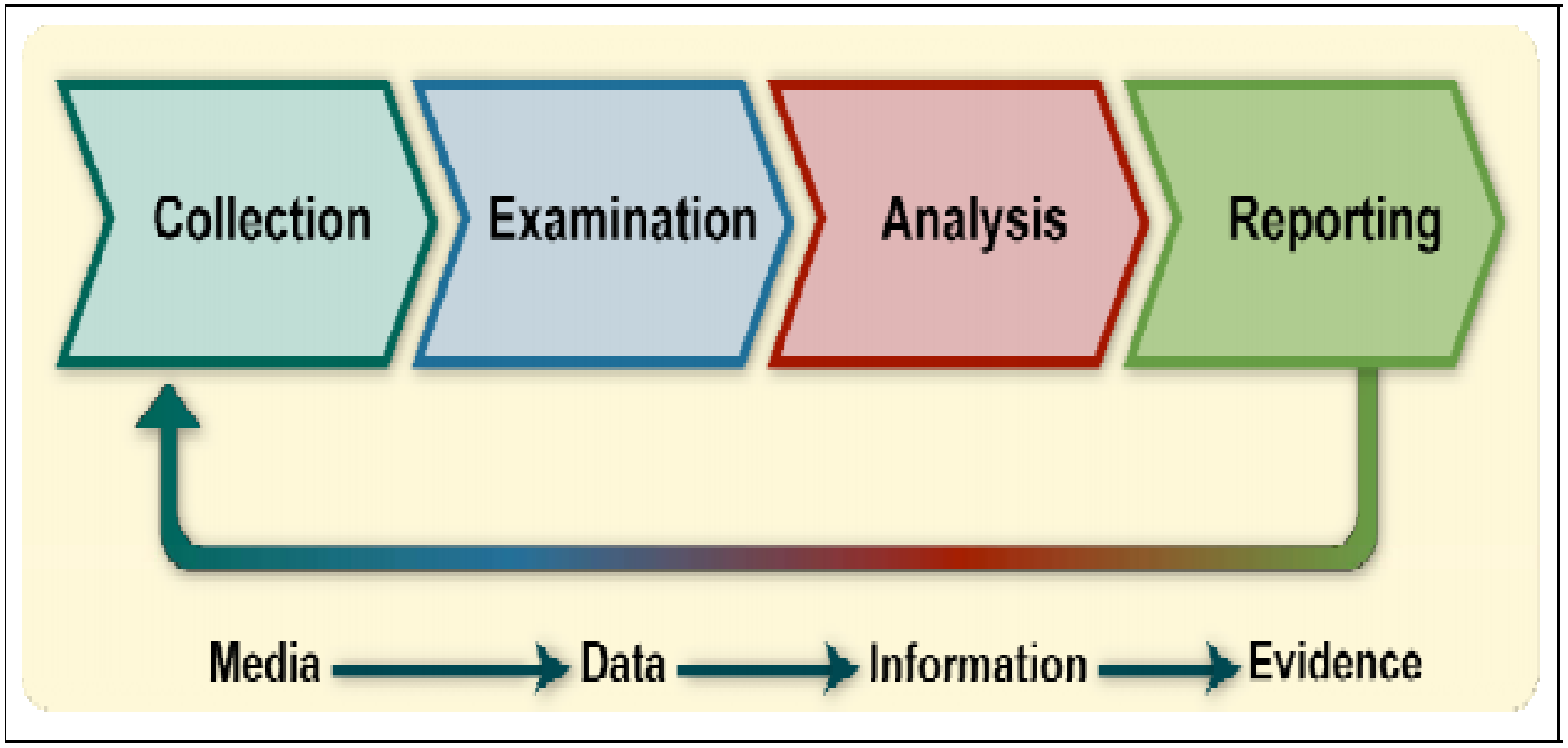
زنجیره حفاظت نمونه و پرونده های ارجاعی به آزمایشگاه

- ارائه شواهد و مدارک مستند جهت ارائه به مراجع قضایی روندی زمان بر است.
- در صورت رعایت کلیه مراحل chain of custody شواهد با سرعت و دقت بیشتری در اختیار سیستم قضایی قرار خواهد گرفت.

Chain of custody

- **Chain of custody** (CoC), in legal contexts, is the chronological documentation or paper trail that records the sequence of custody, control, transfer, analysis, and disposition of physical or electronic evidence.

Chain of custody



Chain of custody

- The chain of custody requires that from the moment the evidence is collected, every transfer of evidence from person to person be documented and that it be provable that nobody else could have accessed that evidence.
- It is best to keep the number of transfers as low as possible.

مشخص بودن پرسنل در کلیه مراحل

- به منظور حفاظت نمونه ها و مستند سازی کلیه مراحل گردش نمونه در آزمایشگاه لازم است کلیه پرسنل دخیل در مراحل نمونه برداری، نقل و انتقال نمونه، انجام آنالیز و ارائه پاسخ آزمایشگاه مشخص بوده و کلیه مراحل مستند شوند.
- نقل و انتقال کمتر نمونه ها حفاظت بیشتر آن ها را تامین می کند.

Chain of custody form

CHAIN OF CUSTODY FORM

(Also fill out detailed site and sample information on either the Lake or Stream Water Sampling Record Form)

Forest / Wilderness / Park / Other (Circle One) **Name:** _____ **Contact Individual and Affiliation:** _____ _____	**Shipped to (Lab Name and Address):** _____ _____ _____	**Page ____ of ____**
Address _____ _____ **Phone Number** _____	**Lab Phone #** _____ **Lab Contact** _____ **Lab Email** _____	**Shipped by: UPS/ FedEx/USPS/Other** _____ **Shipping #** _____

Date Sampled	Time Sampled (24-hour) ☐ Standard ☐ Daylight Savings	Sample ID (Barcode)	Sample Location Lake/Stream Name or Latitude/Longitude	Sample Type (Normal, Rep 1, Rep 2, Blank, Split)	Filtered (Y/N) Where? (Field or Field Lab)	Preserved (Y/N/Type)	Analyses Requested	Lab ID Assigned
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Comments:

Received/Relinquished by:

Print Name	Signature	Date & Time Relinquished	Date & Time Received

Received at Laboratory by:

Print Name	Signature	Date Received	Time Received

- The responsibility of forensic toxicology laboratory is to detect and identify drugs and poisons in postmortem and non-biological samples.

- At least six different test groups are required to be analyzed to exclude even the most commonly encountered poisons.

Group Tests

- *Group 1: Gases*
- *Group 2: Volatile substances*
- *Group 3: Metals*
- *Group 4: Drugs*
- *Group 5: Pesticides*
- *Group 6: Miscellaneous substances*

Why searching for drugs and poisons?

- TDM
- STA
- Detect drugs and substances of abuse
- Postmortem Drug Testing
- Workplace Drug Testing
- Identification of Controlled Substances or Drugs

Deaths Investigated by Toxicologists

1. Accidental Poisonings
2. Drug Abuse Cases
3. Drug Facilitated Crime

   Suicidal Poisonings

   Homicidal Poisonings

   Incapacitate victims of kidnapping, robbery, or sexual assaults

Postmortem Forensic Toxicology

- Qualitative and quantitative analysis of drugs or poisons in biological specimens collected at autopsy.

Toxicological Analysis of Tissue

- Collect biological samples from organs and tissues.
- A forensic toxicologist cannot simply look for the presence of a toxin or drug in a body, she must understand how the body processes these molecules (pharmacokinetics & toxicokinetics).
- Toxicological analysis must start as soon as possible after a person's death.

Sample & Sampling for Toxicological Analysis

Why multiple organs for sampling?

- When collecting the specimens, many different body fluids and organs should be collected since drugs have different affinities for body tissues and therefore multiple extractions (for specific analyses) may be needed.

Issues in Specimen Collection

- **Selection**
  - Multiple, varied sites of collection
- **Collection**
  - Appropriate method of collection
  - Adequate volumes for analysis
- **Storage and handling**

Types of samples

- The specimens available for analysis may be numerous, they include:
- Biological Samples
- Non-Biological Samples

Postmortem biological samples

1- Stomach Content (all available)
2- Blood (20-30 mL, 10 mL)
3- Urine (all available)
4- Liver (250 gr)
5- Kidney
6- Vitreous Humor (all available)
7- Bile (all available)
8- Muscle (10 cm^3)

Biological Specimens

Urine

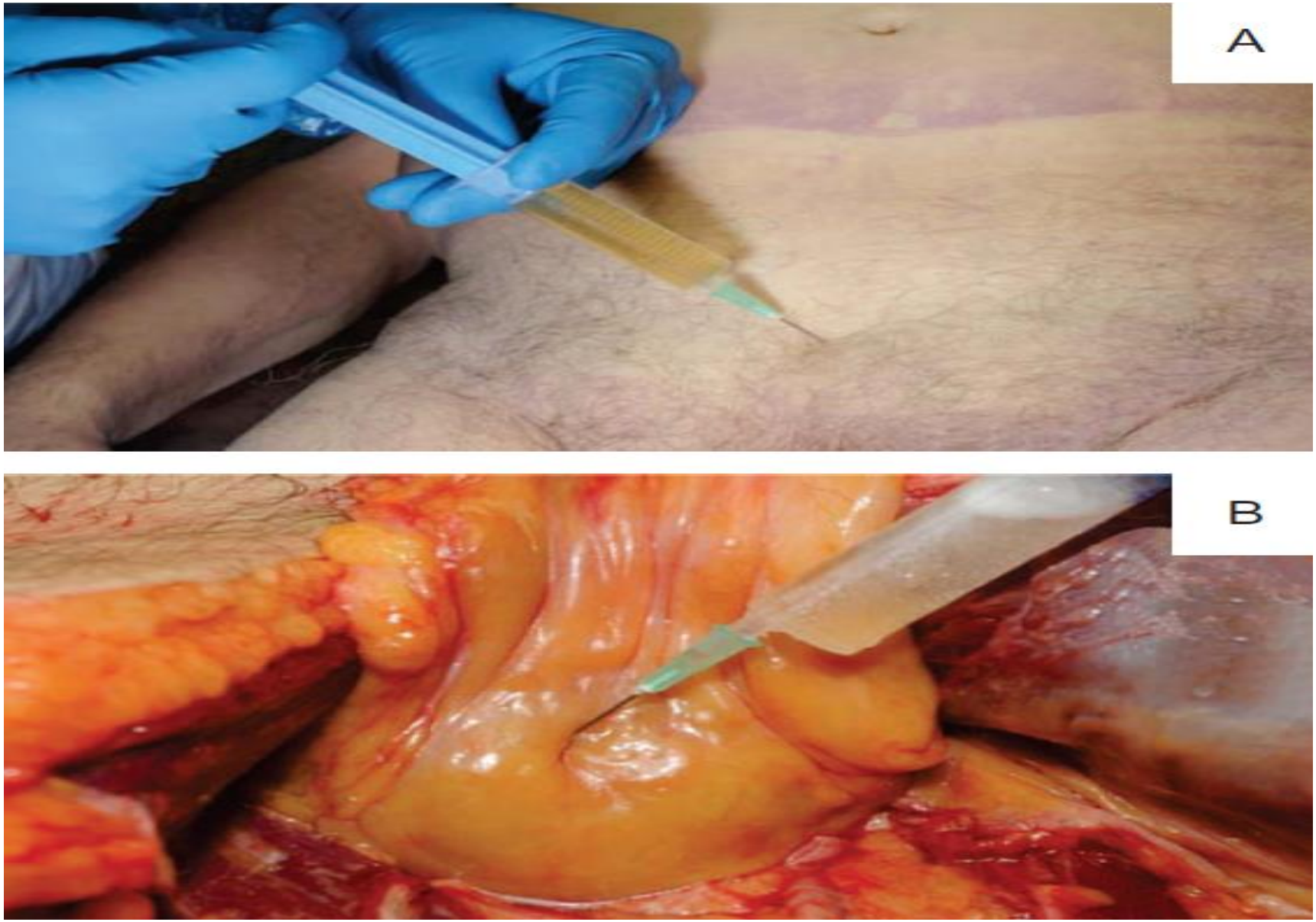
- Produced by the kidneys
- Blood filtered by the kidneys
- Stored in the bladder until voided

- Qualitative - the presence of a drug in the urine of an individual indicates that some time prior to death the drug or poison was present in the blood of the individual.

Urine

1. It contains higher concentrations of drugs and poisons metabolites.
2. It is available in large volumes.
3. In man more than 90% of an administered dose of morphine is excreted in the urine.
4. Only about 10% is unchanged morphine, and M3G is the major metabolite.

Postmortem urine collection



Evaluation of urine samples

- **Adulterating**, **substituting**, and **diluting** urine samples are common practices used to avoid detection of drug use.
- Understanding specific characteristics of a urine specimen can help in identifying false-negative results.

Evaluation of urine samples

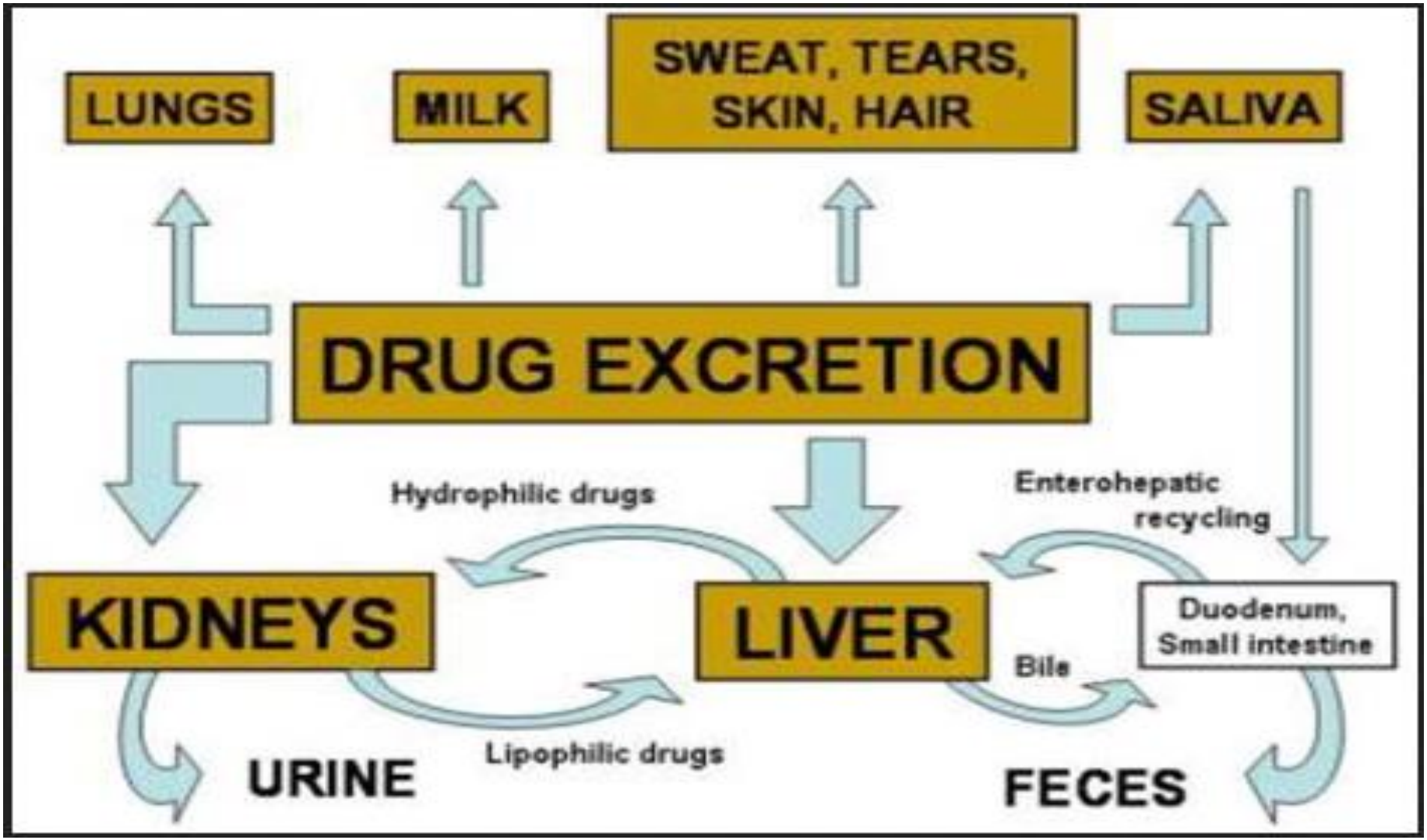
- The first step in evaluating a urine sample is documentation of the **appearance and color.**
- Urine specimens should be shaken to determine whether such substances as **soap** have been added to the urine.
- **Excessive bubble formation** that is long lasting can indicate an attempt to adulterate the specimen.

Common Adulteration Methods

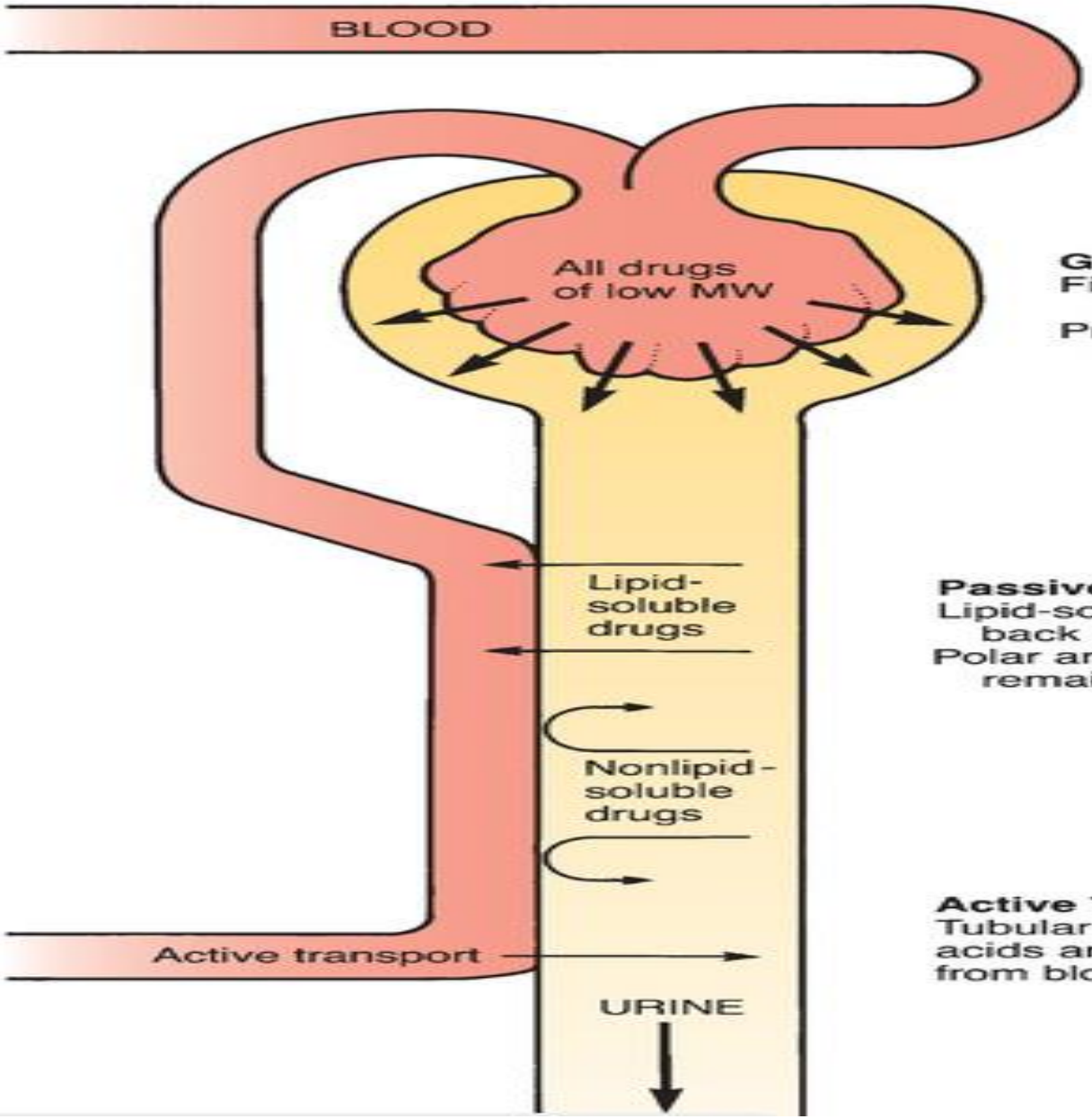
- 1. Substituting "clean" or ***"drug-free urine"*** for drug- positive urine.
- 2. Adding ***adulterants*** such as table salt, bleach, liquid soap, sodium hydroxide, lemon juice, vinegar, Potassium nitrate, Potassium permanganate
- 3. ***Diluting*** the urine specimen with water.

- 4. Ingesting ***acidic salts*** (e.g. Vit. C, ammonium chloride) substances to hasten the excretion of basic drugs ***before*** the urine collection.
- 5. Ingesting ***basic salts*** (e.g. sodium bicarbonate) to reduce the excretion of basic drugs ***during*** collection.
- 6. Ingesting substances that dilute the urinary concentration of the drug to be detected (e.g. drinking water, diuretics).

Drug excretion



دفع کلیوی داروها



Glomerular Filtration
Filtration moves drugs from blood to urine
Protein-bound drugs are not filtered

Passive Reabsorption
Lipid-soluble drugs move back into the blood
Polar and ionized drugs remain in the urine

Active Transport
Tubular "pumps" for organic acids and bases move drugs from blood to urine

حذف داروها از راه ادرار

- در فرایند excretion یا حذف، اشکال دست نخورده دارو و یا متابولیت های آن ها از راه های دفعی بدن مانند ادرار از بدن خارج می شوند.
- pH ادرار، pH خون و خصوصیات ساختاری دارو در میزان حذف آن از راه کلیه موثرند.

قاعده کلی

- داروهای قلیایی (مانند آلکالوئیدهای تریاک و بسیاری از داروها که در علم سم شناسی اهمیت دارند) در pH اسیدی به فرم **یونیزه** (نمک محلول در آب) تبدیل می شوند و برعکس.
- داروهای اسیدی (مانند استامینوفن، فنوباربیتال، استیل سالیسیلیک اسید یا آسپیرین) در pH قلیایی به فرم **یونیزه** (نمک محلول در آب) تبدیل می شوند و برعکس.

مرفین آلکالوئیدی با ساختار قلیایی

Amine (or aniline)

$R_3N:$

NOT IONIC

soluble in ether
not soluble in water

$H^+ Cl^-$

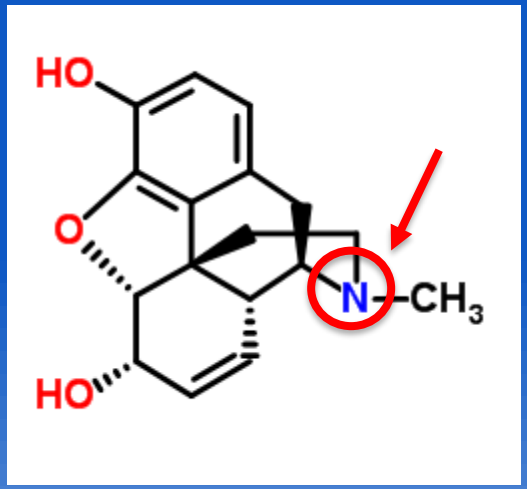
pH اسیدی

Ammonium (or anilinium) ion

$R_3\overset{\oplus}{N}-H$ + Cl^-

De-protonated form:
IONIC

not soluble in ether
soluble in water

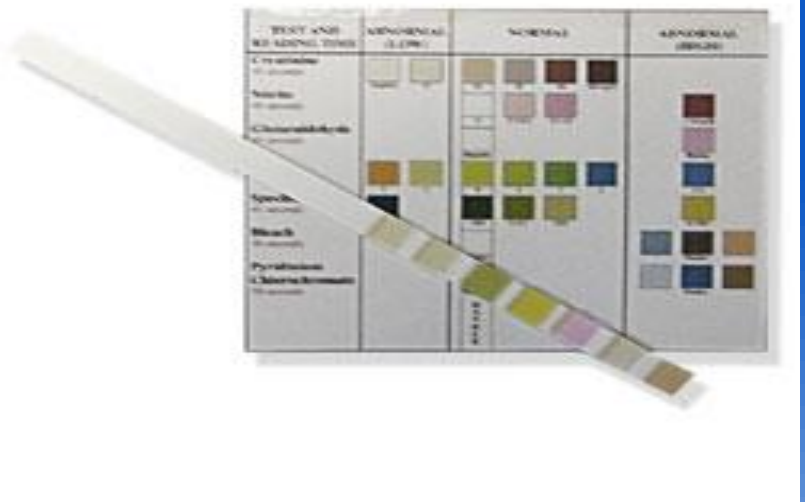


ساختار شیمیایی مرفین

pH of urine samples

- The pH for normal urine fluctuates throughout the day but usually is in the range of 4.5 to 8.0.
- Specimen contamination should be suspected if the pH level is less than 3 or greater than 11 or if the specific gravity is less than 1.002 or greater than 1.020.

Urine Testing: The Most Commonly Used Drug Test



Stomach contents

- A portion of the stomach contents are typically collected at autopsy.
- Stomach contents may contain unabsorbed poisons, tablets, capsules, caplets which may be intact and visible.
- These can be removed from the stomach contents, and identified.

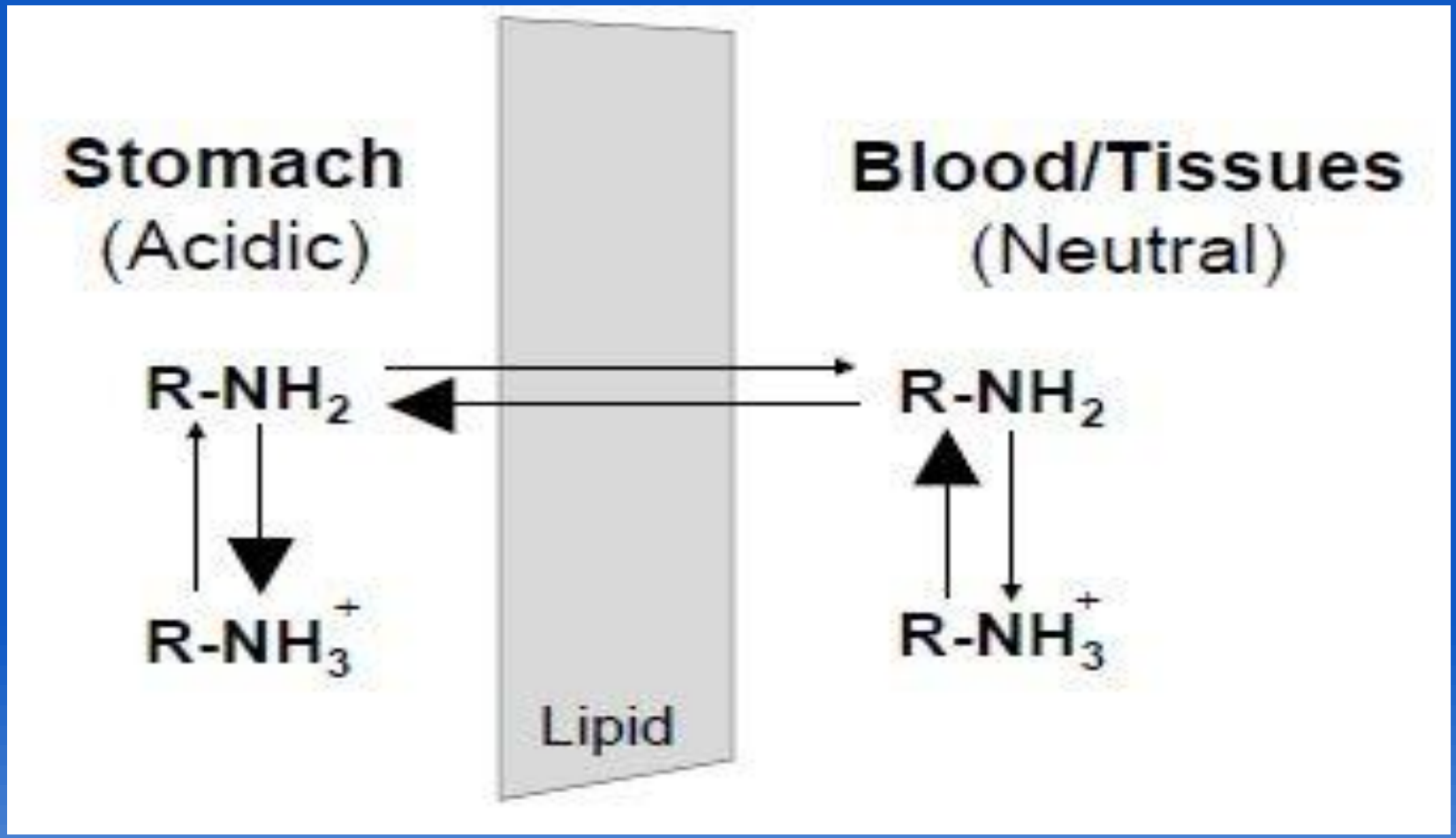
Stomach contents

- ***Drugs in the stomach contents do not necessarily indicate oral ingestion.***
- Many weak bases are subject to secretion into the gastric contents due to the pH gradient between plasma and the stomach.
- Ion trapping in the stomach produces very high concentrations of these agents, **even after intravenous or other non-oral routes of administration.**

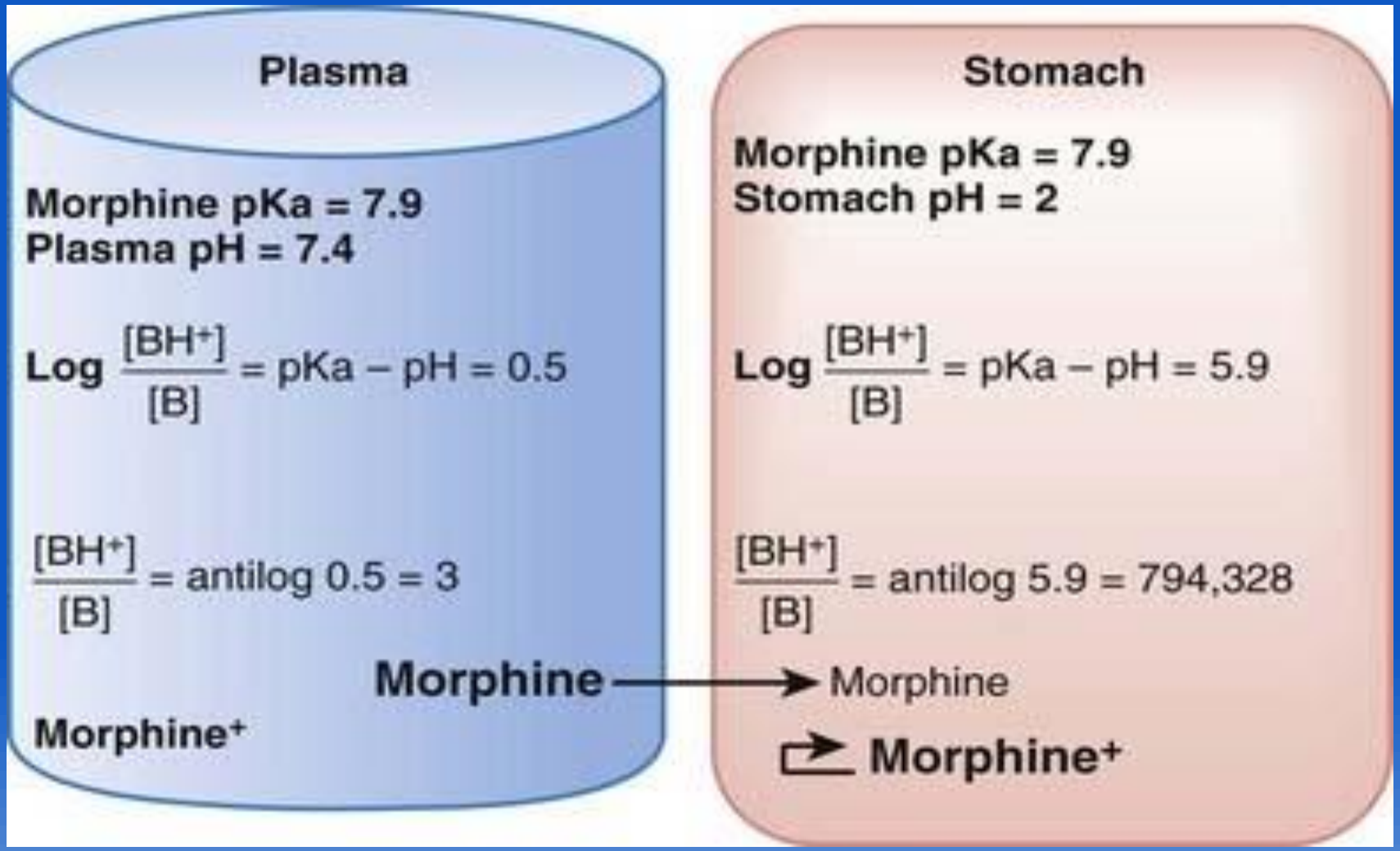
Ion Trapping phenomena

- In cell biology, **ion trapping** is the build-up of a higher concentration of a chemical across a **cell membrane** due to the **pKa** value of the chemical and difference of **pH** across the cell membrane.
- This results in **basic chemicals** accumulating in acidic bodily fluids such as the **cytosol**, and **acidic chemicals** accumulating in basic fluids such as **mastitic milk.**

Ion trapping for drugs



Ion trapping of morphine



Important note

- Finding of even appreciable amounts of a basic drug in the gastric contents is not a reliable indication of oral ingestion.

Can you quantify stomach contents to determine if a person has taken a drug overdose?

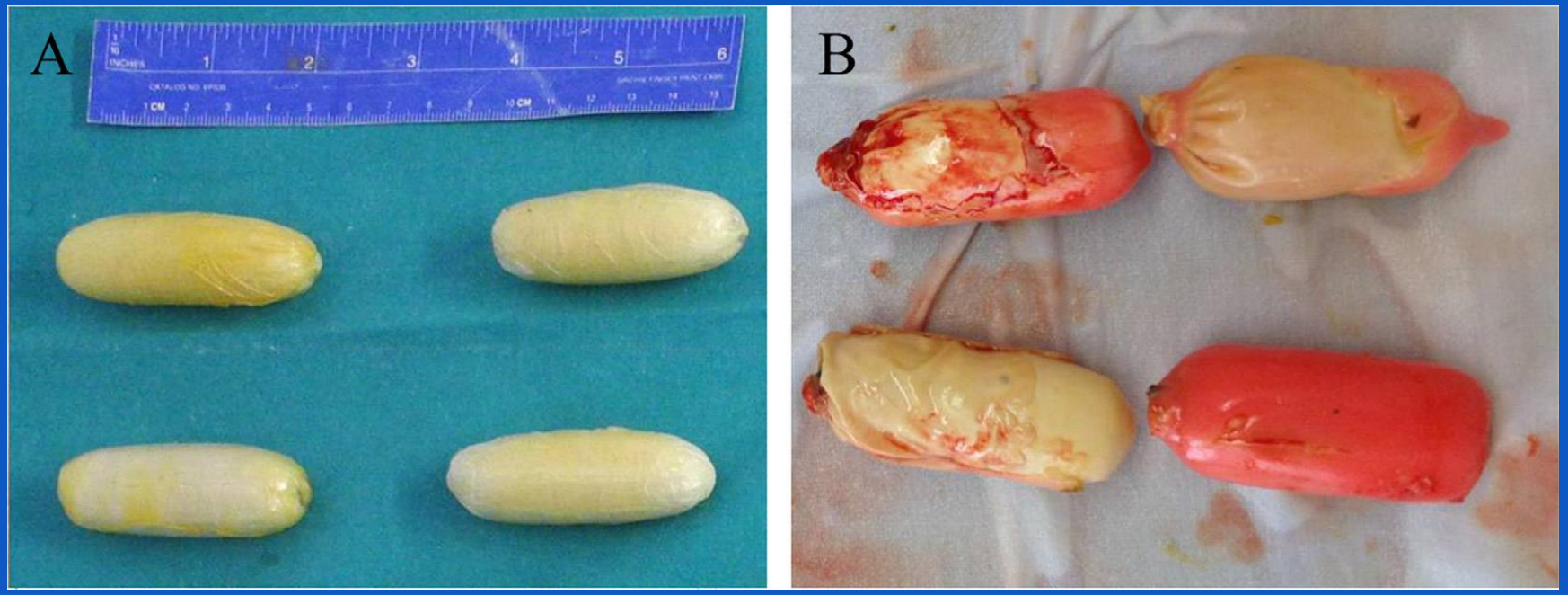
- Stomach contents are not homogenous in their nature.
- Stomach contents are typically a scooped, non-homogenous sample, it is not acceptable practice to quantify the contents and make any valid interpretation from the results.

Stomach contents

1. It may include vomit, gastric aspirate and stomach washing.
2. Intact capsules & tablets in the stomach content are good samples for toxicological analysis.
3. Body packers hide packages of cocaine, heroin, … in body cavities or swallow them.
4. This is typically done in a sudden death in which the decedent has large quantities of a lethal agent in his stomach.

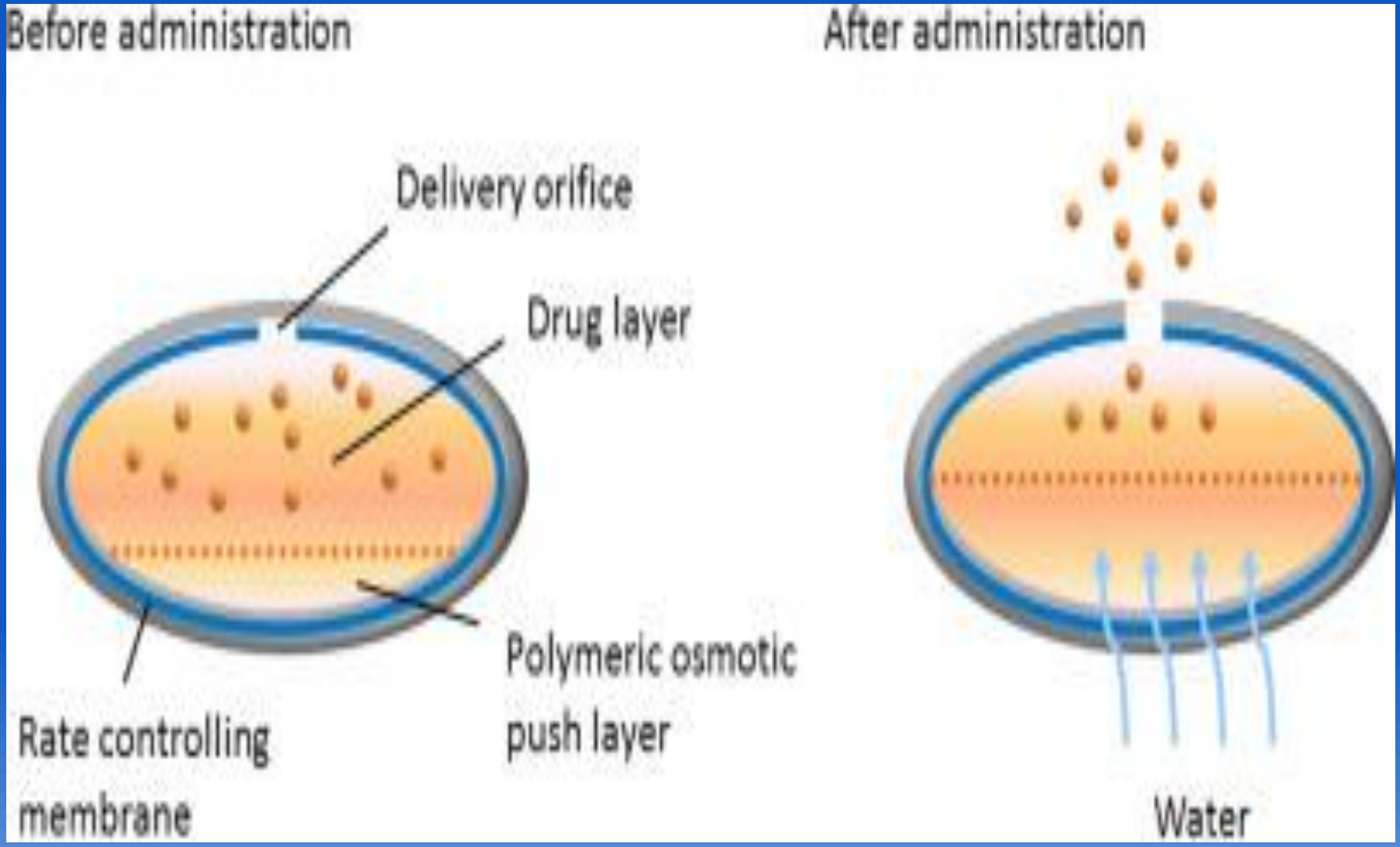
Drug packages in gastric content

Drug packages in gastric content



Tablets in gastric contents

Sustain release tablets in stomach content



Bile

- The liver filters the blood and is most heavily involved in drug metabolism.
- Bile is a digestive secretion that is continuously produced by the liver and stored in the gallbladder.
- Since it is produced by the liver, it is similar in respect to the liver samples in that concentrations of drugs in the bile are usually greater than concentrations in the blood.

Bile

- Therefore the duration of detection of a drug may be increased in the bile compared to the blood.
- As with any quantitative analysis, however, the presence of a drug in the bile simply indicates that sometime prior to death the individual was exposed to the drug.

Bile

- Toxins are concentrated by the liver and excreted into the gall bladder.
- Direct collection of bile into a bottle is advised because bile is too viscous to be sucked through a needle.
- Because bile drains from the liver it is often very rich in certain types of drugs such as opiates and opioid glucoronide metabolites.

Liver

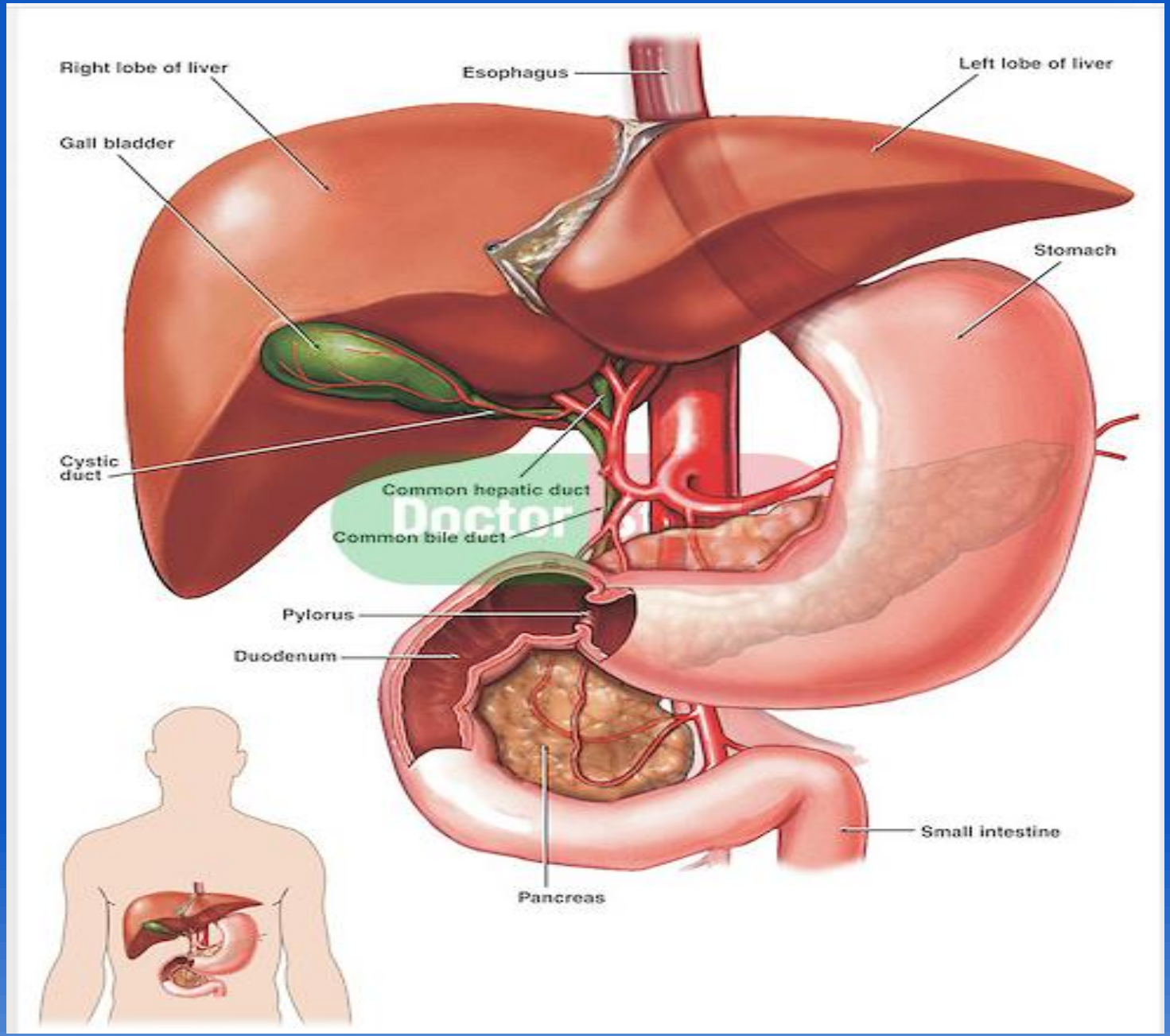
According to it's structure, which contains metabolizing enzymes and fat, It is the best tissue sample for toxicological analysis.

Liver

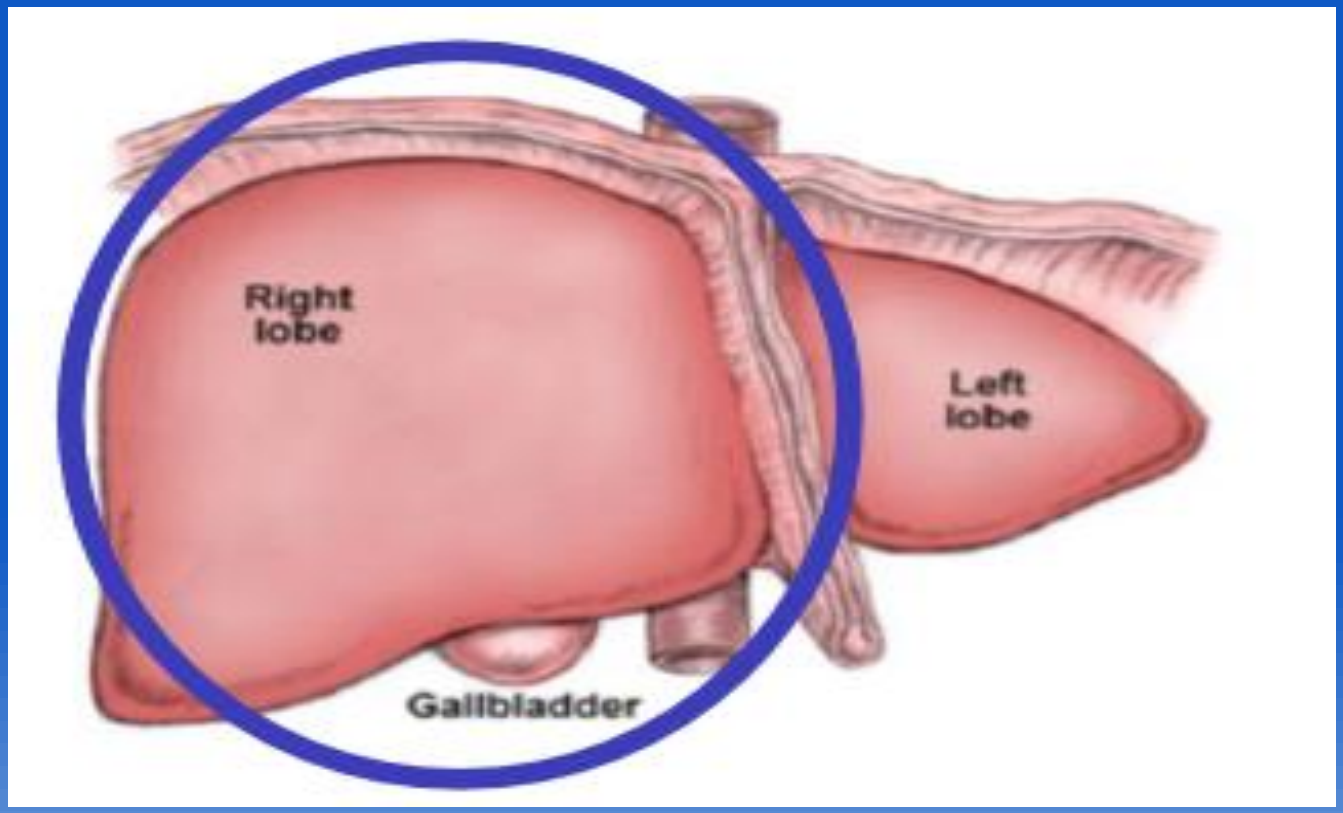
- Liver is the main drug metabolizing organ. As such, most drugs and poisons that enter the blood stream will travel to the liver for biotransformation.
- Both parent compounds (the drug that is ingested) and their metabolites may not only be present in the liver, but may also be present in higher concentrations than in the blood – this may help to identify drugs that are present in quantities that are below detection limits in the blood.

Quantitative analysis of liver

- Quantitative analysis of liver can be performed, however it is very difficult to make an interpretation with any certainty from liver analysis.
- Drugs are not uniformly distributed throughout the liver and therefore, where you sample the liver for drug analysis may have an effect on the quantity of drug that is identified.

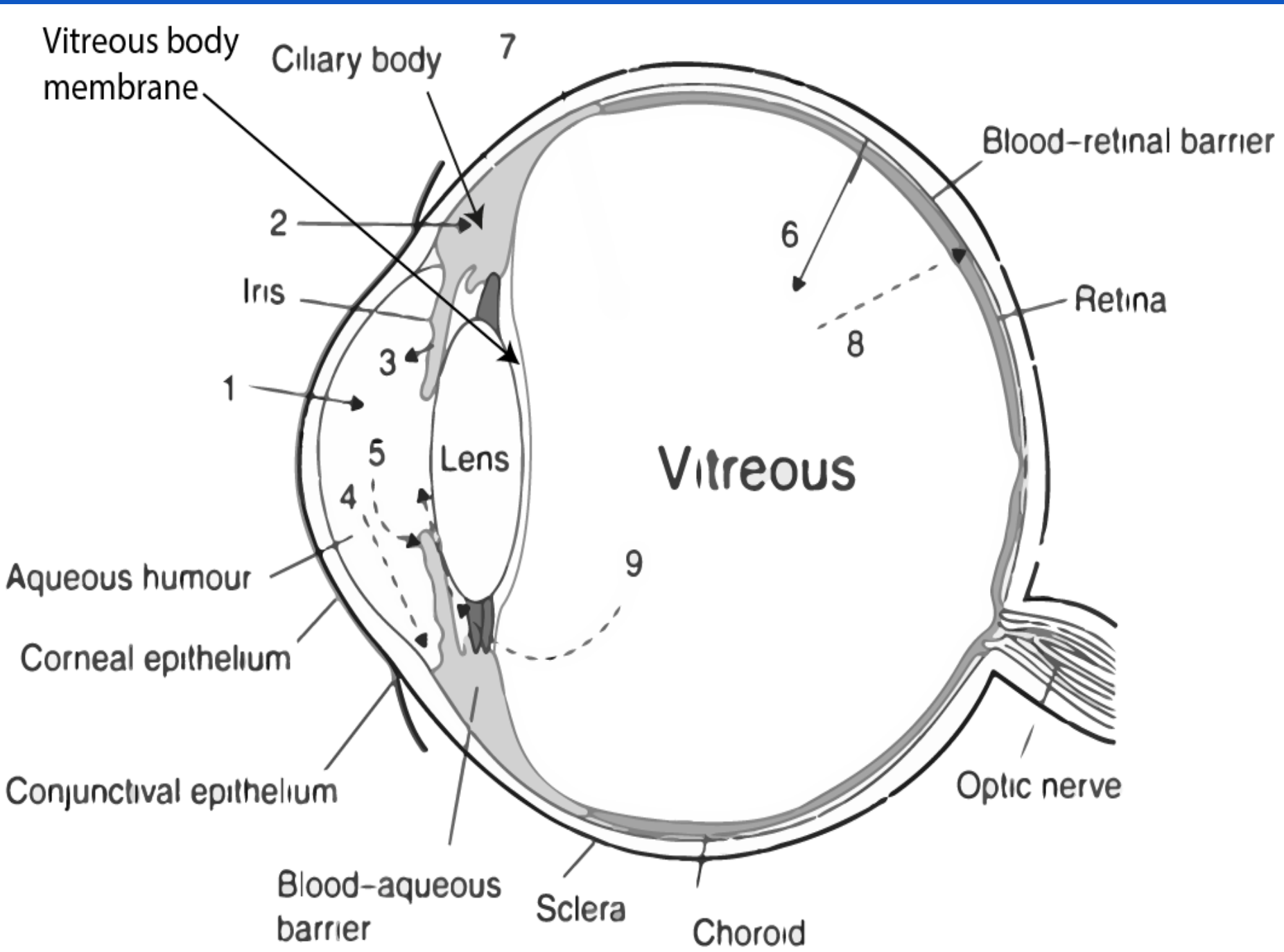

Right lobe of liver
Esophagus
Left lobe of liver
Gall bladder
Stomach
Cystic duct
Common hepatic duct
Common bile duct
Pylorus
Duodenum
Small intestine
Pancreas

Deep right lobe of liver is preferred to avoid contamination with diffusion of drugs from gastric contents into the left lobe (postmortem redistribution)



Vitreous humor

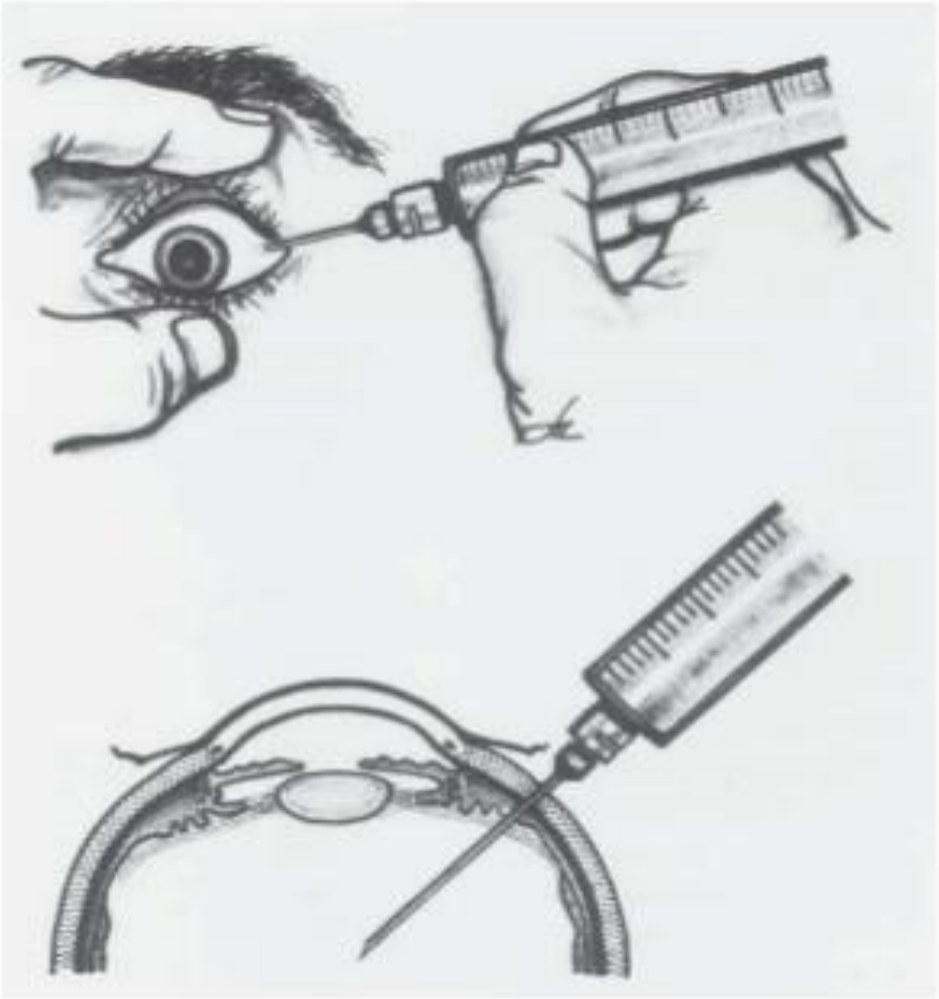
- Fluid that occupies the space between the lens and the retina of the eye.
- Vitreous humor is in a protected position behind the lens of the eye.
- Because of this protected position, it is isolated from putrefactive processes, from charring and from trauma.

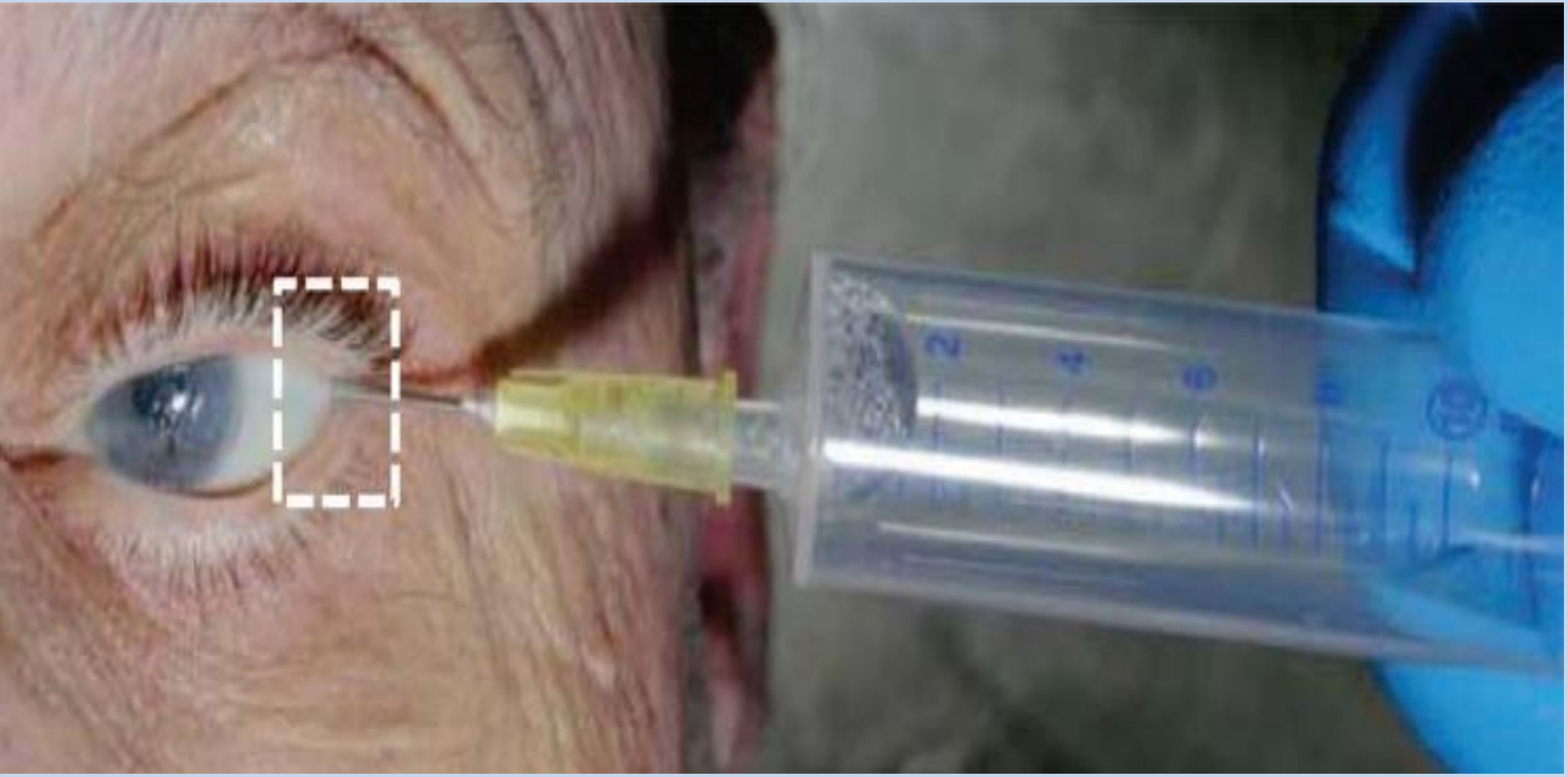

Vitreous body membrane
Ciliary body
7
Blood–retinal barrier
2
6
Iris
Retina
3
8
1
5
Lens
Vitreous
4
9
Aqueous humour
Corneal epithelium
Conjunctival epithelium
Optic nerve
Blood–aqueous barrier
Sclera
Choroid

Vitreous humor

- For example, the vitreous humor can be obtained intact even if a corpse has been extensively burned or damaged.
- Blood is very susceptible to postmortem changes.
- Vitreous fluid is less susceptible to these effects, particularly because it is likely to be free from microorganisms.

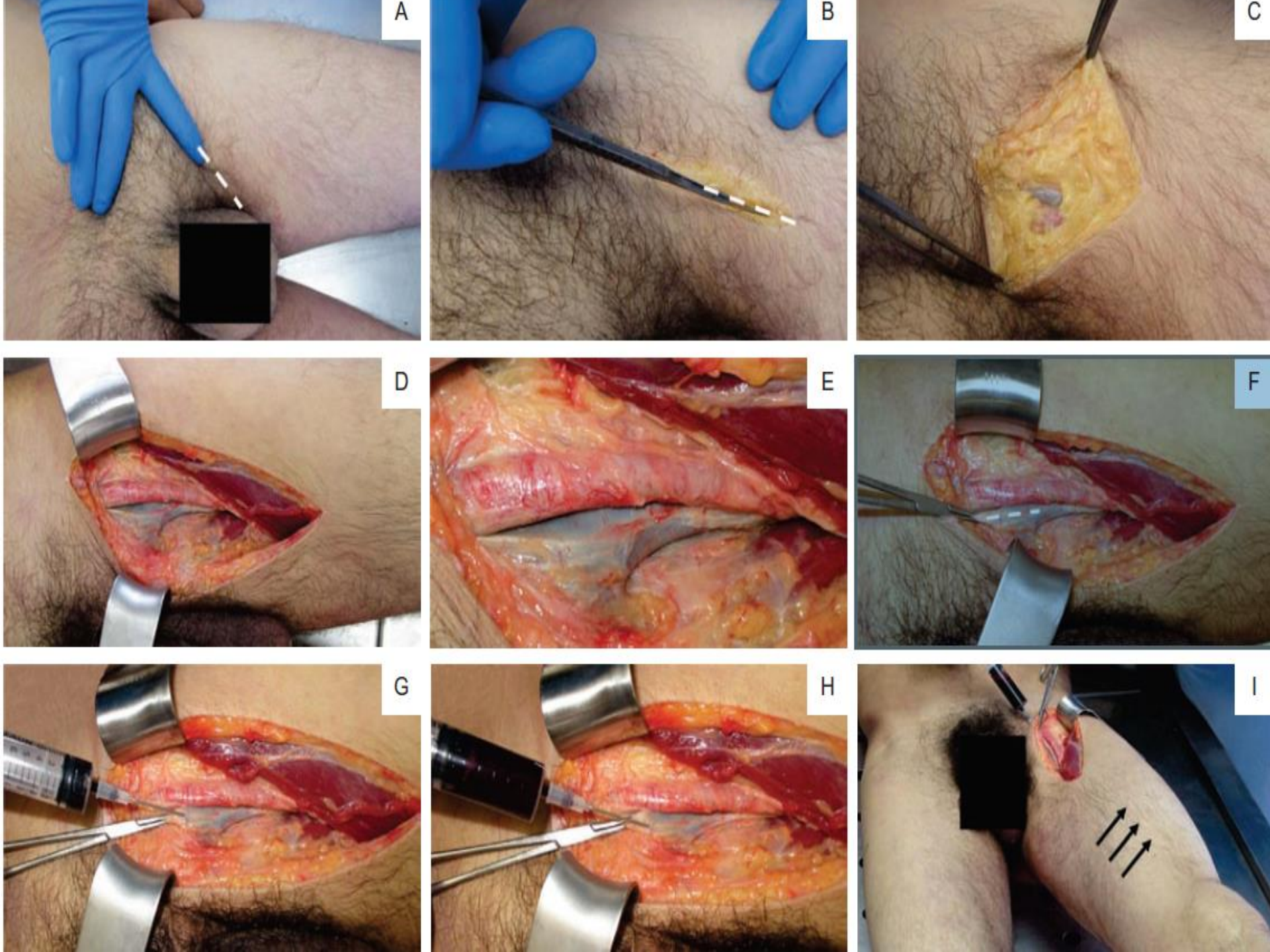
Aspiration of vitreous. Upper, Needle inserted 5 mm lateral to the limbus (corneo-scleral junction). Lower, Needle enters vitreous through pars plana of ciliary body.





Blood

- Peripheral blood concentration have been shown to be more reliable for toxicological analysis than the conventional heart blood.
- ***Sodium fluoride*** protects blood from postmortem changes such as bacterial production of ethanol or other alcohols.
- It also helps to protect other labile drugs such as cocaine, nitrazepam and clonazepam from degradation.


A
B
C
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I

Blood

- Many species of bacteria, yeast, and fungi have the ability to produce ethanol and other volatile organic compounds in postmortem specimens.
- The potential for postmortem ethanol formation complicates the interpretation of ethanol-positive results.
- The prevention of ethanol formation at all steps following specimen collection is a priority.
- **Sodium fluoride** is the most commonly used preservative for postmortem specimens.

Fluoride and enolase activity

- The fluoride ion is seemingly effective in inhibiting the activity of several kinds of enzymes, such as **enolase** a component in the glycolytic pathway, and is important for the action of yeasts, fungi and many micro-organisms responsible for fermentation.

Blood preservation

- Ethanol formation was virtually eliminated when specimens were mixed with **2% W/V** sodium fluoride (NaF).
- There are published reports concluding that sodium fluoride may be ineffective for the prevention of ethanol formation in blood samples containing sufficiently high concentrations of C. albicans.

Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International 165 (2007) 10–29
Forensic Science International
www.elsevier.com/locate/forsciint

Review

Interpreting results of ethanol analysis in postmortem specimens: A review of the literature

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Injection sites

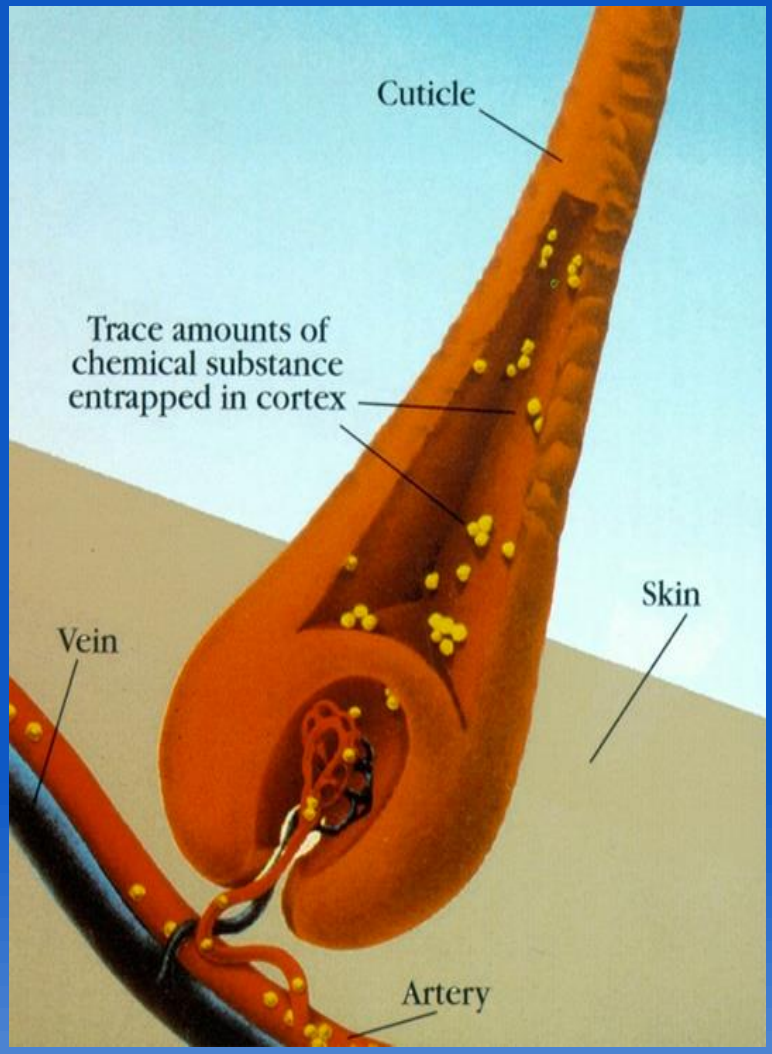
- In case of death due to injection of drugs the sample from the injection site has to be preserved.
- The skin sample with the underneath muscle tissue around the injection site area with a control sample of similar composition from the opposite normal site.

Hair

- Hair is useful sample for analyzing *chronic use of drugs/substances*.
- Hair sample is used to distinguish between episodic or continuous exposure/use (Segmental Analysis).
- 50-100 mg (20-50 hair) from scalp or posterior vertex with the entire root, shaft and tip.

Why hair?


Month 1
Month 2
Month 3
Month 4
Month 5
Month 6
No Drugs
No Drugs
No Drugs
Drugs
Drugs
No Drugs


Cuticle
Trace amounts of chemical substance entrapped in cortex
Skin
Vein
Artery

Hair

- Since hair grows at a predictable rate (generally 1 cm/month) can be used to provide a historical record of drug or poison exposure.
- Procedure is to chop a hair sample into 1 cm increments and analyze them separately to "track" drug exposure over a long period of time.

Sample collection before embalming

- Specimens should be collected before applying processes that may destroy evidence, that is, before embalming.
- The process of embalming, for example, may destroy or dilute the drugs and may yield a false positive result, for example, for the presence of methanol (which is a constituent of embalming fluid).

Samples taken after embalming

- Typically embalming fluid contains a mixture of formaldehyde, methanol, and other solvents.
- **Methanol** is a typical component of embalming fluid.
- Most drugs are soluble in methanol.
- Embalming process will essentially “wash” the vasculature and tissues.


STERILOL
PURITY
SUPREMOL
EMBALMING
FLUID
QUALITY
RECTIFIANT
WATER
CORRECTIVE
CONCENTRATED
Hydrol
BLEACHOL
GLASS THIS SIDE UP WITH CARE
HYDROL
EMBALMING
FLUID

Biological samples in alive persons

- Urine and blood samples should be collected as soon as possible in suspected opioid and alcohol abuse.

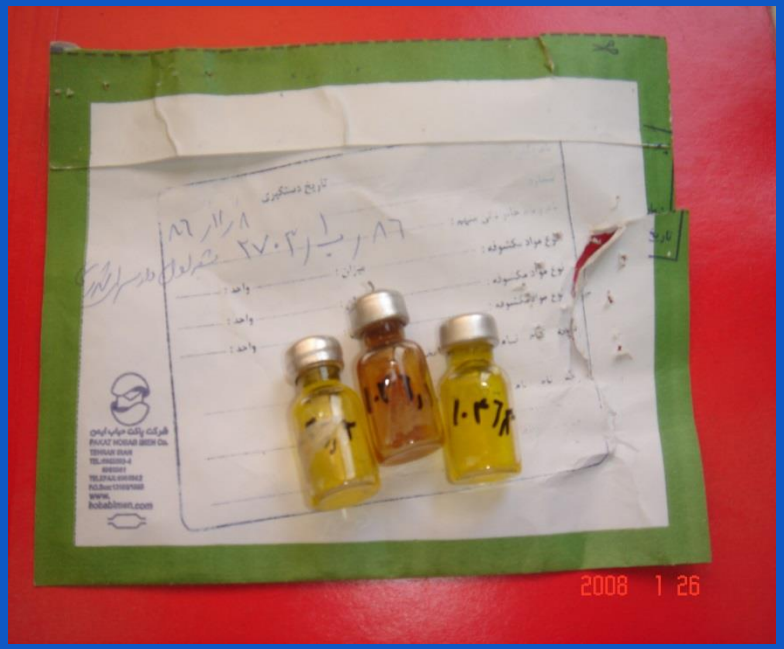
Non-biological Specimens

Non-biological submissions

- Used to direct analysis of biologicals
- May indicate the nature of substances that may have been ingested, inhaled or injected
- Examples:
  - Containers found at the scene
  - Syringes
  - Unidentified tablets or liquids

Non biological samples

- Tablets
- Ampoules
- Powders
- Capsules
- Drugs of abuse
- Syringes
- Food & Beverage Residues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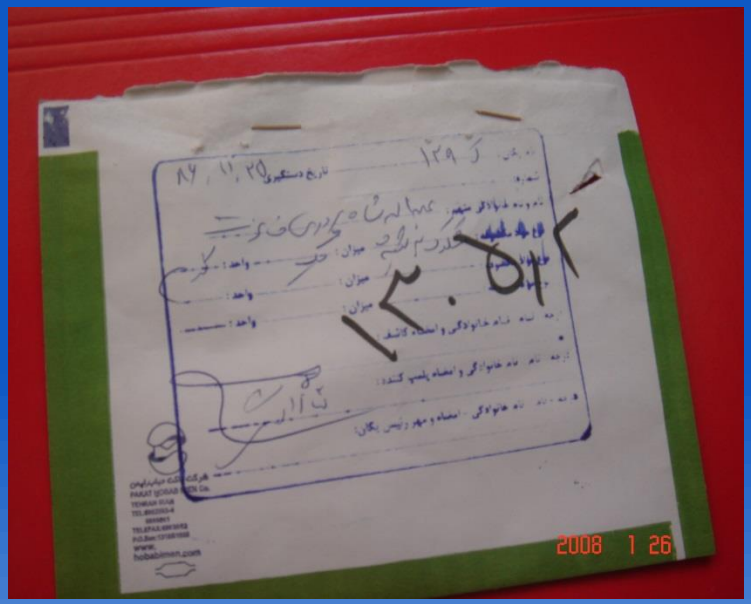
2008 1 27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PINEAPPLE DRINK
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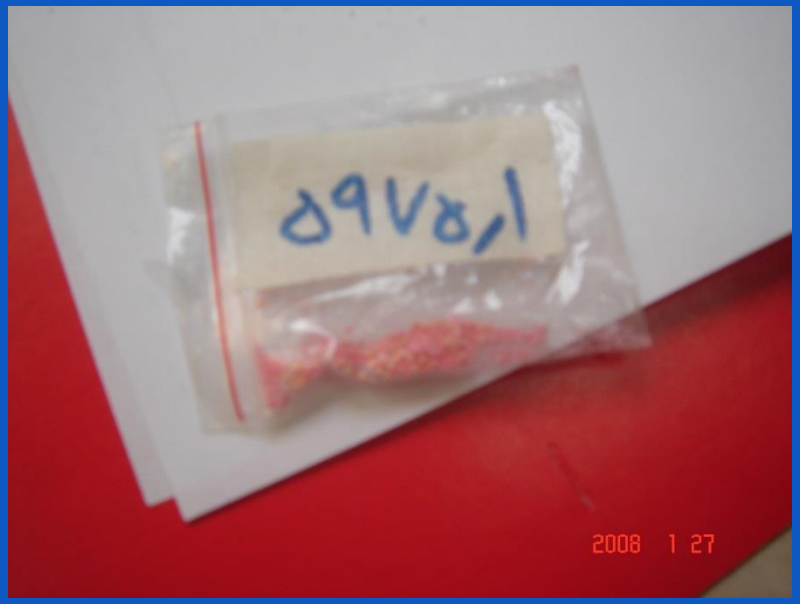
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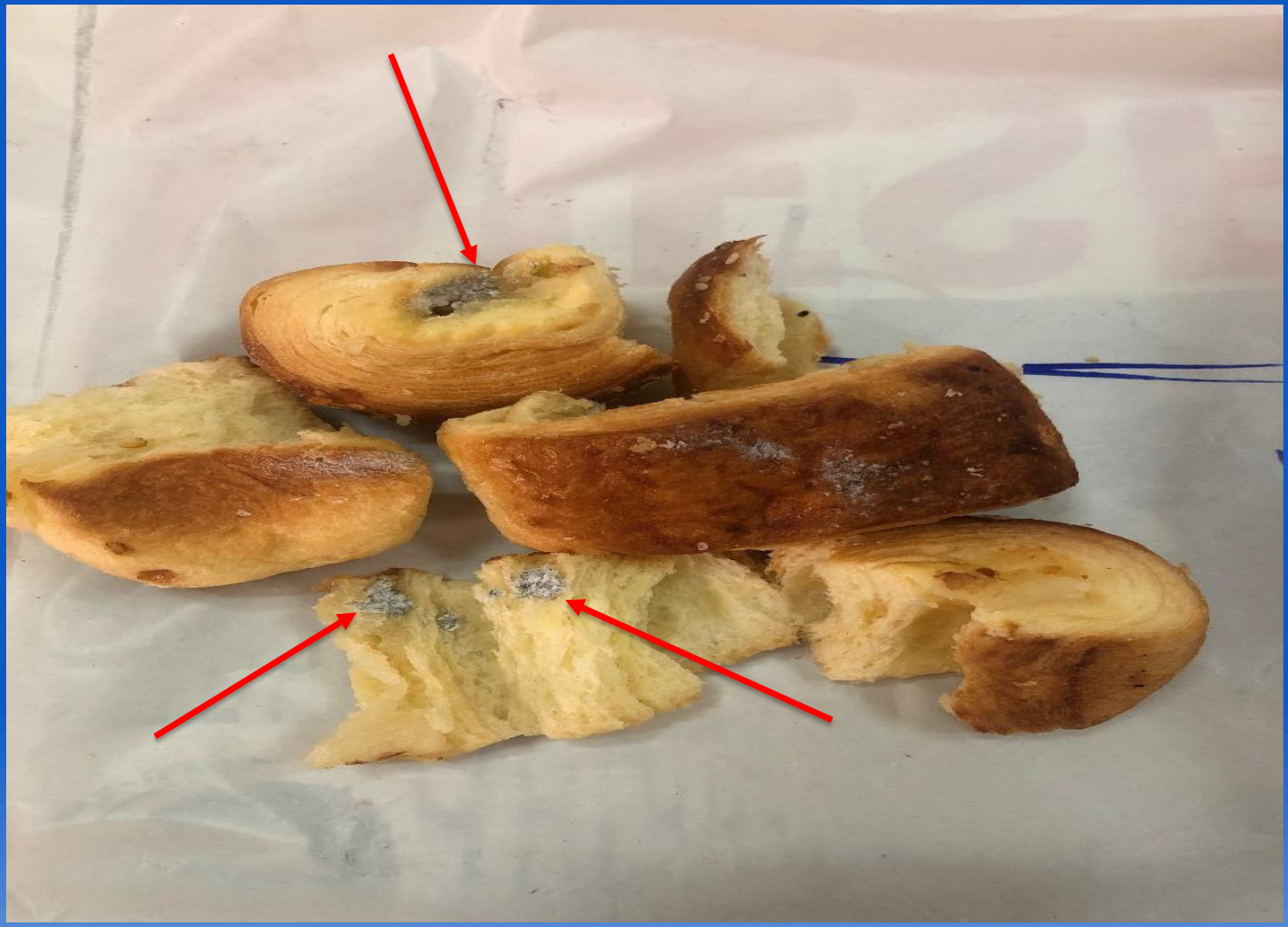
١٢٤٢/٢
١٢٤٢/١
١٢٤٢/٣
2008 1 27


ABSOLUT
KURANT
١٢٤٢/٣
١٢٤٢/٢
EXTRA SPECIAL WHISKY
GLEN GRAND
BLENDED WHISKY
١٧٠
١٧٠
2008 1 27


2008 1 27


2008 1 27

شیرینی آلوده به آلومینیوم فسفید



مواد مورد سوء مصرف و اشکال آن ها

ماده	اشکال موجود
آمفتامین	پودر، کپسول، قرص
هروئین	پودر
تریاک	رزین، پودر
LSD	قرصهای رنگی ریز، بلوک های ژلاتینی، کاغذ آغشته
کوکائین	پودر سفید برفی، کلوخه
حشیش	برگ خرد شده گیاه، رزین، روغن
بنزودیازپین ها و باربیتورات ها	قرص، کپسول، پودر، محلولهای تزریقی

هروئین

	 **ADAM**	 **EVA** 130 mg	 **Amor** Bruchrille	 **Love** Herz	 **Herz** Bruchrille	 **Drops** Bruchrille	 **Sonne** Bruchrille	 **Halbmond** Bruchrille	 **Herzpfeil** Bruchrille	 **VW** Bruchrille
 **Käfer**	 **Mercedes**	 **Triple Five**	 **V.I.P.**	 **CAL** Bruchrille	 **PT** Bruchrille	 **Schlitzauge** Bruchrille	 **ANADIN**	 **Boomerang**	 **Bulls** Bruchrille	 **Delphin** Bruchrille
 **Elephant** Bruchrille	 **Hund** Bruchrille	 **Pigs** Ringelschwanz	 **Pelikan** Bruchrille	 **Taube** Bruchrille	 **Friedens-taube**	 **Spatz** Bruchrille	 **Vogel**	 **Kermit** Bruchrille	 **Feuerstein** Bruchrille	 **Batman**
 **Superman**	 **Popeye** Bruchrille	 **Chiemsee** Bruchrille	 **Fido** Bruchrille	 **Häuptling** Bruchrille	 **Sonic** Sonic/Bruchrille	 **Smiley** SMILE	 **Playboy** Bruchrille	**Schwalbe**	**Dino** Bruchrille	**Anker** Bruchrille
Pilz Bruchrille	**Olympics**	**Hammer& Sichel**	**Gorbys** CCCP	**Kleeblatt** Kleeblatt	**Kleeblatt** Bruchrille	**Liebessymbol** Bruchrille	**Yellow Shunshine**	**Pink Panther**	**Snowball**	**Ying Yang**

قرص های اکستازی

آمفتامین و متامفتامین

Determining analyses

- Case history
  - *Medical history*
  - *Autopsy findings*
- Experience of the toxicologist
- Amount of specimen available
- Nature of specimens available
- Policies of the organization

Pitfalls in Postmortem Forensic Toxicology

Decomposition

- Autolysis
  - The breakdown of cellular material by enzymes
- Putrefaction
  - A septic/infectious process
  - The destruction of soft tissues by the action of bacteria and enzymes
  - Traumatic deaths may demonstrate ↑ putrefaction

Decomposition

- Fewer samples available for collection
- Quality of samples is diminished
- Putrefaction produces alcohols
  - Ethanol
  - Isopropanol
  - Acetaldehyde
  - *n*-propanol

Postmortem redistribution

- Postmortem redistribution (PMR) refers to the **changes that occur in drug concentrations after death**.
- It involves the **redistribution of drugs into blood** from solid organs such as the lungs, liver, and myocardium.
- Drug properties such as volume of distribution, lipophilicity, and pKa are important factors.

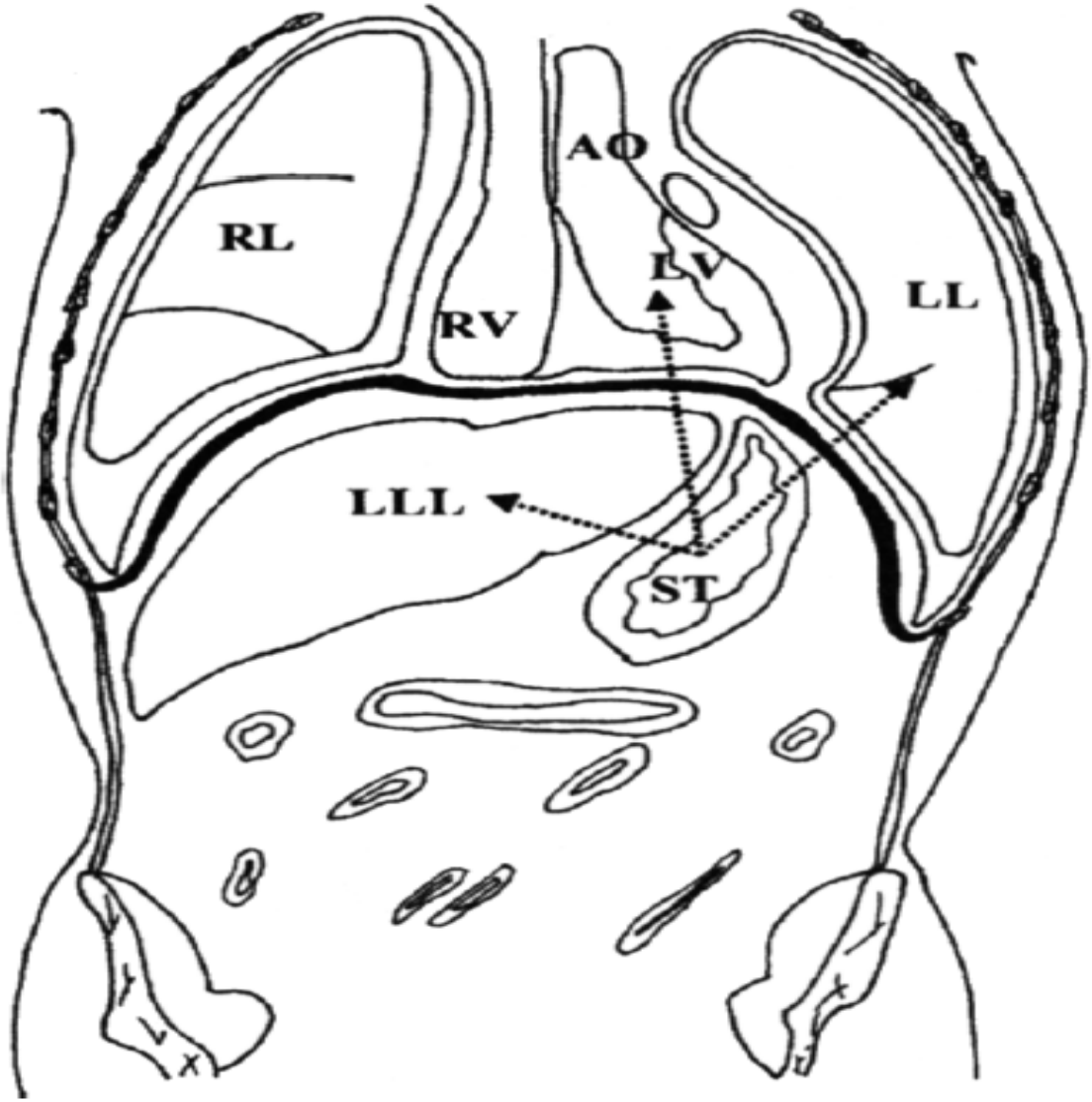
Postmortem redistribution

- A phenomenon whereby increased concentrations of some drugs are observed in postmortem samples and/or site dependent differences in drug concentrations may be observed.

- Typically central blood samples are more prone to postmortem changes (will have greater drug concentrations than peripheral blood samples).

Possible mechanisms of postmortem redistribution

- Diffusion from specific tissue sites of higher concentration (e.g. liver, myocardium, lung) to central vessels in close proximity
- Diffusion of unabsorbed drug in the stomach to the heart and inferior vena cava
- Diffusion of drugs from the trachea, associated with aspiration of vomitus



Frontal view of thorax and abdomen through the left ventricle. The arrows show the direction of redistribution towards the main organs concerned (RL, right lung; LL, left lung; AO, aorta; RV, right ventricle; LV, left ventricle; ST, stomach; and LLL, left liver lobe).

Postmortem redistribution

- Basic, highly lipophilic drugs with a volume of distribution greater than 3 L/kg are most likely to undergo PMR.
- Examples include the tricyclic antidepressants, digoxin, and the amphetamines.
- The anatomical location of blood sampling can influence the drug concentration.
- The ideal site is a ligated or clamped femoral vein.

Susceptible Drugs

Drugs most commonly associated with postmortem redistribution:

1. Are chemically basic
2. Have large volumes of distribution (Vd) 

Volume of distribution

- Volume of distribution is the amount of drug in the whole body (compared to the amount of drug in the blood).
- If a drug has a large volume of distribution, it is stored in other fluids and tissues in the body.
- The volume of distribution (Vd), also known as apparent volume of distribution, is a pharmacological, theoretical volume that the total amount of administered drug would have to occupy (if it were uniformly distributed), to provide the same concentration as it currently is in blood plasma.

حجم توزیع

- حجم توزیع معادل حجمی فرضی از بدن است که دارو اشغال می کند تا اینکه غلظتی معادل غلظت پلاسمایی به شکل یکنواخت در بدن ایجاد شود.

Susceptible Drugs

- Tricyclic antidepressants
  - Amitriptyline
  - Nortriptyline
  - Imipramine
  - Desipramine
- Antihistamines
  - Diphenhydramine
- Narcotic Analgesics
  - Codeine
  - Oxycodone
  - Propoxyphene
- Doxepin
- Digoxin

Example: Digoxin

- A 33 year old white female was admitted to hospital after taking 60 digoxin tablets
- An antemortem blood sample collected 1 hour prior to her death indicates a blood digoxin level of 18 ng/mL
- Heart blood digoxin concentration obtained at autopsy is 36 ng/mL

Example: Digoxin

- Postmortem increase in blood digoxin concentrations is suspected to be due to the release of the drug from the myocardium.
  - Postmortem levels > Antemortem levels
  - Heart blood levels > Femoral blood levels

Drug Stability

- Knowledge of a drug's stability is necessary to facilitate interpretation of concentrations
- Breakdown of drugs may occur after death and during storage via non-enzymatic mechanisms
  - Cocaine → Benzoylecgonine (Hydrolysis)
  - LSD → degradation due to light sensitivity

Fluoride and refrigeration help to prevent the conversion to derivatives.

Evaporation of volatiles

- Ethanol
- Carbon monoxide
- Cyanide
- Toluene
- Other alcohols

Example: Carbon Monoxide

Ocak et al. 1985. J. Analytical Toxicology. 9: 202-206

- Effects of storage conditions on stability of CO
  - No significant change in % CO saturation in capped samples stored at room temperature or 4°C
  - Significant losses in % CO saturation in uncapped samples stored at room temperature and at 4°C
- Mechanism for loss → diffusion

Interpretation and reporting of analytical toxicology results

- It is important in forensic pharmacology and toxicology to be aware of the extent to which samples can be interpreted on the basis of the known *pharmacology of a drug*.

Two-Step Testing Approach

- Screening test – designed to separate negative samples from samples that are "presumptively" positive

- Confirmation test – follow-up procedure designed to validate positive test results

  - distinctly different analytical technique
  - more *specific* and more *sensitive*

- When a biological drug screen is performed, with the intention of detecting a specific drug, there are four possible outcomes:

- 1. True Positive (TP): The result of the test is positive, and the drug is present in the sample.
- 2. False Positive (FP): The result of the test is positive, but the drug is not present in the sample.
- 3. True Negative (TN): The result of the test is negative, and the drug is not present in the sample, or is present below the threshold concentration.
- 4. False Negative (FN): The result of the test is negative, but the drug is present in the sample above the threshold concentration.

Drug tests & cross reactivity

- Screening tests can and do react to “non-target” compounds therefor:
- Obtain list of interfering compounds from lab
- Initial screening (“instant” tests) are only 60-70% accurate
- Confirm positive results

Negative/none detected interpretation

- Client is not using a drug that can be detected by the test
- Client not using enough drug
- Client's drug use is too infrequent
- Collection too long after drug use
- Urine is tampered or adulterated
- Test being used not sensitive enough
- Client using drug not on testing list

Factors Influencing Detection Window

- Drug dose
- Route of entry into body
- Duration & frequency of use
- Rate of metabolism
- Testing sensitivity
- Specificity of testing method

Pharmacokinetics

- Pharmacokinetic processes of:

Absorption

Distribution

Metabolism

Elimination

determine *how rapidly* and for *how long* the drug will appear at the target organ.

Results reporting

- Laboratory reports will provide a qualitative interpretation for the laboratory's specific panel of drugs, based on the testing laboratory's established cut-off concentrations.
- These cut-offs (usually given in ng/mL or µg/L) may or may not be listed on the laboratory report, but are readily available from the laboratory performing the testing.

Items to be mentioned in results reporting

- Date of preparing test request
- Date of admission to laboratory
- Specimen type (urine, blood, etc.)
- Type of analytical technique used (immunochromatography, TLC, HPLC and GC/MS)
- Analysis result (analyte found: methamphetamine, amphetamine, methadone,…)

Interpretations of drugs of abuse test results

- Patient-specific factors such as weight, dose, level of hydration, time lag between drug ingestion and sample collection, can influence the amount of drug excreted in the urine.

Pharmacokinetic properties, such as the **half-life** of the substance being detected and the individual rate at which the substance is metabolized by the patient, will also affect the ability of the tests to detect the drug(s) in question.

Concluding remarks

- Choosing the best anatomic site and the best samples are among the important factors to get reliable results from forensic toxicology laboratories.

- Post mortem redistribution should be considered in the decision making about route of drug exposure, its dose and other pharmacokinetic properties.