Introduction to Samples and Sampling for Clinical Toxicological Analyses

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Introduction

The selection of the correct samples for analysis and their preservation are prerequisites in the investigation of poisoning

Applications of Clinical Toxicological Analysis

- Confirmation of diagnosis
- Differential diagnosis of coma
- Diagnosis of brain death
- Influence on active therapy
- Medicolegal aspects of hospital toxicology

Sampling Procedures

1. Choice of samples and sample collection
2. Packaging of samples
3. Labeling of samples
4. Transportation of samples
5. Storage of samples

Choice of Sample and Sample Collection

- Type of drug/poison (pharmacokinetic behaviors)
- Route of administration
- Time of administration

Type of Samples

1. Stomach Content:

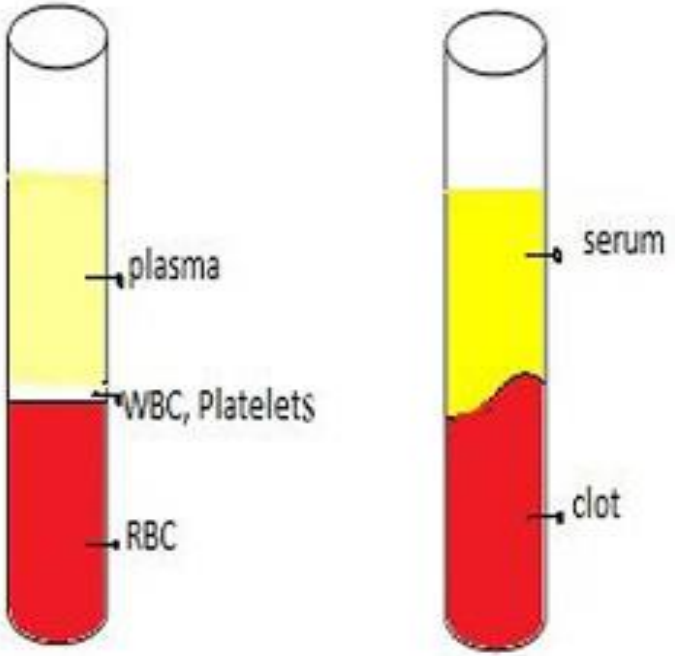
- Stomach content, stomach washing, vomit and vomit on clothing.
- These samples are essential for use in general screening tests.
- A negative result in the screening procedures may indicated that the drug was not taken orally. (Exceptions??)
- Smuggling & Basic drug secretion into the gastric juice.

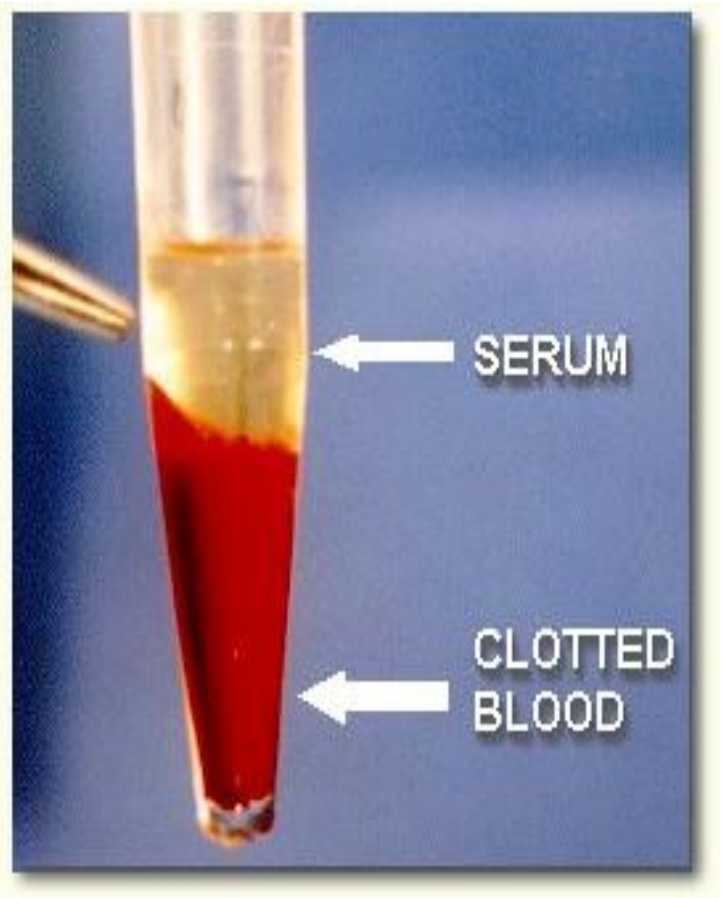
- Stomach washout is no longer a routine treatment procedure, but when it is carried out it is important to obtain the first sample of washout rather than a later sample, which will be diluted considerably.

- If the sample is obtained soon after the overdose, it may be possible to recognize the presence of undegraded tablets and capsules, or the characteristic odor of certain compounds.
- Stomach contents can be substituted for urine in toxicological screening, and are useful for identifying poisons derived from plants and fungi, and for other poisons that are difficult to detect in blood or urine.

2. Blood

- This is the most useful sample for analysis in clinical toxicology.
- Blood samples: Whole blood, Plasma, Serum
- ✓ Advantages and limitations
- Blood Sample Preservation (NaF 1.5- 2% w/v)


plasma
WBC, Platelets
RBC
serum
clot


SERUM
CLOTTED
BLOOD

- A 10 mL sample of anticoagulated blood (Sodium Edetate) and 10 mL of clotted blood should be collected from adults on admission (proportionately smaller volumes from young children).
- Most quantitative assays are carried out on the plasma, but anticoagulated whole blood is essential if the poison is associated mainly with the red cells (e.g. carbon monoxide, cyanide, lead, mercury).
- Serum from coagulated blood can also be used, although the levels are almost always the same as those in plasma.
- Serum has the advantage that there is no potential interference from any additive.

- It is advisable in addition to collect a 2mL blood sample into a fluoride/oxalate tube if ethanol ingestion is suspected.
- However, since most of the fluoride tubes used in hospitals do not contain enough sodium fluoride to completely inhibit microbial production of alcohol (the minimum fluoride concentration required in blood is 1.5% w/v), these samples are not acceptable for forensic purposes.

3.Urine

- *Advantages:*

1.Concentration of a drug in the urine may be about 100 times higher than in the blood.

2. It is free from proteins with a consequent low background of interference.

3. Non- invasive sampling procedure.

4. Suitable window detection

- *Disadvantages:*

1. Some drugs are excreted as metabolites by this route(e.g.THC, Benzodiazepines,..)
2. In acute intoxication cases (e.g. Injection of opioids or HCN inhalation), may be no drug was detected in urine.
3. Susceptible for manipulation

- Urine usually contains higher concentrations of drugs, poisons and their metabolites than blood and is therefore ideal for qualitative screening.
- Most drugs remain detectable for much longer periods in urine than in blood. For example, GHB will have almost entirely disappeared from blood by the time a patient reaches hospital but can often still be found in a urine sample.
- Some substances are detectable only as a metabolite in urine (e.g. Oxaxepam as oxazepam glucuronide; nicotine as cotinine).

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- However, the detection of a substance in urine does not necessarily mean that this is the cause of the poisoning since it may have been taken days before the event and may not be related to the acute situation.
- In emergency cases, particularly when the patient is unconscious, the delay in obtaining a urine sample may be unacceptable and many clinicians are now reluctant to use catheterisation routinely on unconscious patients.
- Where a sample can be obtained, a volume of <25mL is sufficient for most purposes.

Saliva

- Advantages:
- 1. Drug concentrations in saliva reflect the free, unbound parent drug and lipophilic metabolites circulating in the blood. Since these are the forms of the drug which cross the blood-brain barrier and effect performance and behavior, saliva is a good specimen for detecting drug involvement in driving behavior or impairment of performance.
- 2. The collection of saliva is simple and non-invasive.

- Disadvantages:

  1. It has short detection window (as blood) in comparison with urine and hair.

  2. Saliva may not be available from al individuals at all times.

Oral Fluid (Saliva) Testing

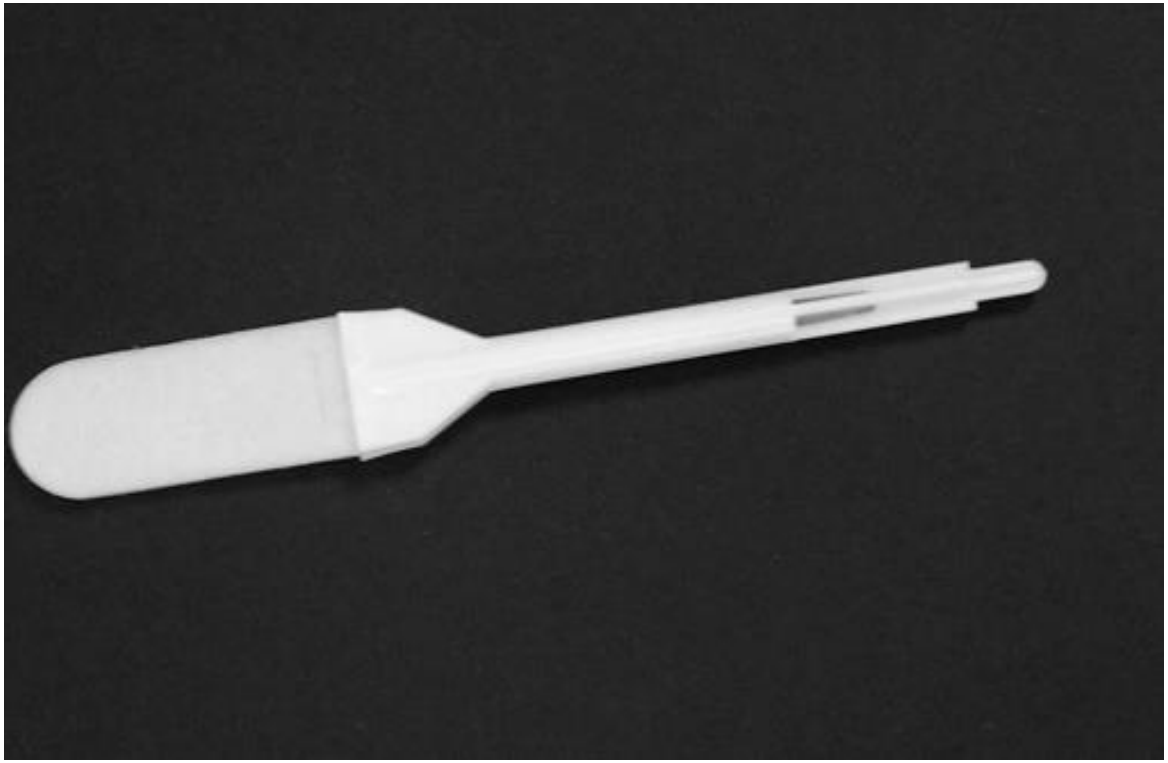
Main features/advantages…

- Non-invasive collection
- Easy to supervise
- No adulteration or substitution
- Short windows of detection*
- Recent drug use
- Oral Fluid testing detects drugs that are in the blood at the time of testing
- Detects many drugs in the parent form eg: d9-THC

Oral Fluid (Saliva) Testing

Limitations…

- Volume of saliva is low
- Can be slow to collect on occasion
- Drug levels tend to be lower in saliva
- Short windows of detection*(esp THC)
- Less scientific research/validation available



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).
- **Advantages and limitations**
- Sampling site and Sample amount: 50-100 mg (20-50 hair) from scalp or posterior vertex with the entire root, shaft and tip.

Drugs in Hair Analysis

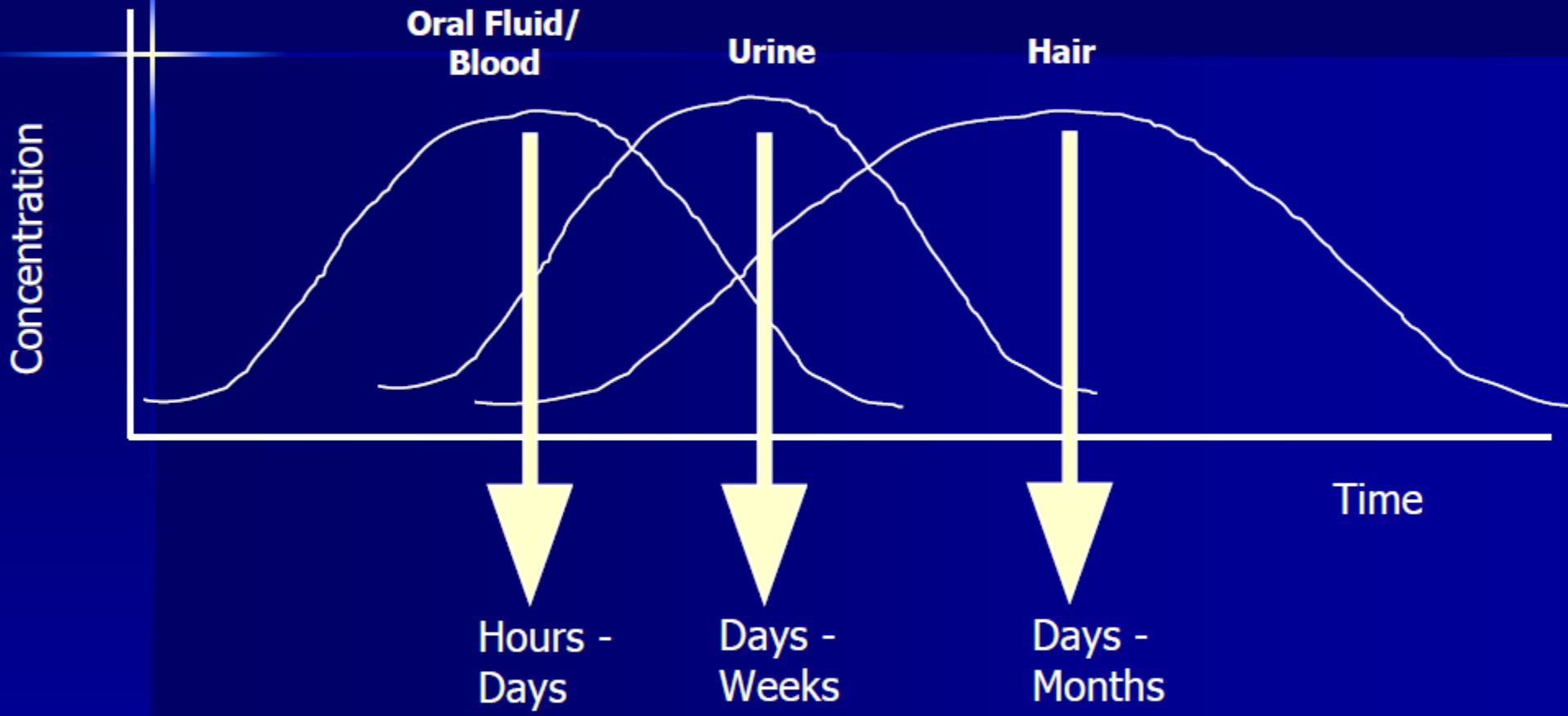
- ATS including: Amphetamine, Methamphetamine, MDMA, MDA, MDEA
- Benzodiazepines and analogues including zolpidem and zopiclone
- Cannabinoids : (THC, Hydroxy-THC, Carboxy-THC)
- Cocaine and associated metabolites
- Opioids (6-MAM, Methadone, Buprenorphine, Pentazocine)
- Anabolic steroids
- Ethylglucuronide (EtG)

- Barbiturates
- FAEE (for alcohol abuse)
- Nicotine
- Digoxin
- Chloroquine
- Heavy Metals (Pb, As, Hg, Cd, Cu,…)

Comparison between Urine and Hair

Parameter	Urine	Hair
Major compound	Metabolites	Parent Drug
Detection period	2-5 days	Weeks,Month
Type of measure	Incremental	Cumulate
Screening	Yes	Difficult
Invasiveness	High	Low
Storage	-20	Ambient temp
Risk of false negative	High	Low
Risk of false positive	Low	ND
Risk of adulteration	High	Low

Generalised Windows of Detection



Please Remember

- **Dose & Route**
- **Frequency of dose**
- **Individual Metabolism**

Packaging of Samples

1. Containers:

- Disposable containers should be used.
- Glass containers for liquid samples (Blood, Urine,..)
- Plastic containers for solid samples(tissues)

- Sealing of containers by PTFE liner.
- Silanised glass tube(For low concentration of drugs < 10 ng/ml)

Labeling of Containers

- Name of patient or ID code, Age, Sex, Date & time of sampling, type of sample, Site of sampling, Biohazards warning, Name of Doctor

- Samples Transportation

Storage of Samples

- **Factors affecting sample decomposition:**

1. Light

- Photo labile drugs (such as: Ergot alkaloids, Phenothiazines)

2. Oxidation

- Phenothiazines, TCAs, Acetaminophen, Morphine, Thiobarbital)

✓ Antioxidants (e.g. Ascorbic acid, Sodium metabisulphite 1% w/v)

3. Temperature:

- ✓ 4°C, -20°C, Freeze- drying of samples

4. Hydrolysis:

- Cocaine, Local anesthetics (Lidocaine)

  (pH< 4 & NaF 1%w/v)

5. Biological Decomposition

- Preservation of samples by preservatives (NaF 1.5%w/v)