



Improving practice: The toxicologist and the pathologist

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The toxicologist and the pathologist: Can we work together?

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I'll show you mine (report) if you
show me yours (report)

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Chain of events

Pathologist
↓
Toxicologist

Toxicologist
↓

1. Death

2. Samples obtained

3. Samples received by laboratory

4. Samples analysed

5. Report produced



In order to assist with deciding on analytical direction and/or eventual interpretation, specific information is required:

- Case Details (deceased's name, DOB, sex, Pathologist, HM Coroner, contact details for any queries)
- Circumstances of the death (i.e. how, when, where)
- Scene evidence (not always obvious)
e.g. OD (tablets) or rapid deaths (syringe in arm), position of the body, suicide notes
- Drug and prescription history (deceased + family – even pets)
- Occupation, hobbies or interests
(may involve access to drugs, chemicals, plants, etc).

Specimens required

- Common biological fluids (e.g. **blood, urine, vitreous humour**)
 - Blood – determines recent drug use in relation to death
 - Urine – determines previous drug use in relation to death
 - Vitreous humour – free from micro-organisms, good for alcohol
- **Stomach contents** (particularly if contains drug material)
 - Can determine drug intake very recently prior to death.
 - Useful in cases that may have an alternative cause (e.g. hanging)
- Tissues (e.g. **brain, lung, liver**)
 - Brain/Lung - particularly useful for volatile substances (e.g. butane)
 - Liver – largely determines previous drug use
- Other matrices (e.g. **hair, bone**) – determines previous and historic drug use
- Seized material (e.g. tablets, liquid, syringes) – provides circumstantial evidence

BLOOD

- femoral vein, collected as to prevent drawing blood from other vessels
- collect in unpreserved container (e.g. 20ml plain universal)
- collect a smaller portion in a fluoride preserved container (e.g. 5ml fl/ox)

URINE

- collect in unpreserved (20ml) and fluoride preserved containers (5ml)


VITREOUS HUMOUR

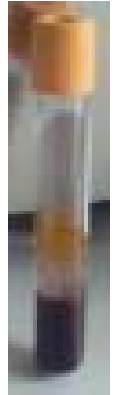
- can be in either unpreserved or preserved containers

All specimens must be appropriately labelled;

- deceased's name
- date of birth
- date and time of collection
- type of specimen (e.g. vitreous can look like urine)
- anatomical site of collection (if blood)

Specimens required

- Use plain (unpreserved) and/or fluoride (preserved) containers with >1% fluoride (not grey tops).
- Try to avoid using “typical” clinical containers for PM samples. Do not use serum/gel containers as the gel can differentially absorb drugs and affect analysis.
- Ensure container lids are secure.
- Write or adhere biohazard warnings (e.g. Hep C, HIV) 
- Refrigerate prior to sending to laboratory and send as soon as reasonably possible. Use appropriate packaging for transport (absorbent material + secondary container + box) UN3373 regs.
- Appropriate chain of custody documentation should be completed.



Specific information and samples are required if a death occurs in hospital.

- If a person has spent some period of time in hospital, whilst alive, the drug is being metabolised all the time so the concentration is reducing and may eventually disappear. Post-mortem specimens are therefore not always appropriate (unless iatrogenic poisoning is suspected).
- Specimens that have been obtained whilst in hospital (ante-mortem) should be retained as soon as possible.
- The date and time of specimen collection should be stated.
- Drugs may have been administered during hospital treatment so this information should also be obtained (e.g. morphine, benzodiazepines).

The type and range of analysis is **laboratory specific** but the process involves sample preparation/extraction and detection/measurement using various techniques.

The approach is also **laboratory specific** and can be guided by case information but should not be confined to this.

Typical screening includes analysis for;

- Chemically basic, neutral and acidic drugs (includes pharmaceuticals, chemicals and drugs of abuse)
- Common drugs of abuse (e.g. cannabinoids, opiates, cocaine, benzos, amphetas)
- Alcohol (multiple samples recommended)

Compounds not always included as part of routine analysis;

- GHB (“Liquid Ecstasy”) Some anti-epileptics (e.g. valproate)
- BHB (marker for ketoacidosis) Metals (arsenic, lead, mercury)
- Paraquat (weedkiller) Volatiles (butane, propane, DCM)
- Ethylene glycol (anti-freeze)

COMMON DRUGS/POISONS AS A CAUSE OF DEATH

1. Heroin/morphine
2. Methadone
3. Alcohol
4. Anti-depressants (dosulepin, amitriptyline, citalopram, venlafaxine)
5. Dextropropoxyphene + paracetamol – far less now
6. Carbon monoxide
7. Cocaine
8. Opiates (codeine) + opioids (dihydrocodeine, tramadol, oxycodone)
9. Volatile substances (e.g. lighter fuel - butane)
10. Barbiturates (amobarbital, secobarbital)

Although death solely due to benzodiazepines is rare, they are one of the most commonly detected drug classes found in fatalities.

UNUSUAL DRUGS/POISONS AS A CAUSE OF DEATH

1. Veterinary products (e.g. etorphine, pentobarbital)
2. Plant poisons (e.g. aconite)
3. Cyanide
4. Strychnine
5. Herbicides (e.g. paraquat)
6. Chemicals/Solvents (e.g. dichloromethane and ethylene glycol)
7. Pesticides/rodenticides (e.g. metaldehyde and warfarin)
8. “Herbal” remedies (that contain pharmaceuticals or heavy metals)



e.g. Yew tree leaf – contains potent taxine alkaloid

Problem compounds for analysis

- There are a number of drugs and poisons that are difficult or not possible to analyse for, particularly in PM samples.
- Can be due to inabilities or problems in extraction and/or detection.

Examples

Chemicals (e.g. bleach), cyanide, gases (e.g. helium, nitrogen, argon), insulin, anabolic steroids (unless by a specialised sports laboratory), some metals, alkyl nitrites (“poppers”), aluminium/zinc phosphide, levodopa, some antibiotics, ACE inhibitors (e.g. enalapril, lisinopril), inhaler medication (e.g. salbutamol), some anti-epileptics (e.g. tigabatin, gabapentin, pregabalin) and newer drugs with no existing analytical data.

Biochemistry and toxicology involve very different methods and analytes. Biochemistry tests are carried out routinely in hospital clinical chemistry departments but typically involve the analysis of in life (ante-mortem) serum or plasma.

- Techniques used are especially designed and validated to be used for AM samples and as such are not generally applicable to PM specimens (especially blood).
- Due to changes after death (e.g. redistribution, autolysis, haemolysis, etc) any PM results can be grossly unreliable or misleading.
- Blood glucose generally falls after death.
- Vitreous humour not usually amenable to routine biochemistry automated assay machines and can produce erroneous results.
- Insulin and C-peptide instability requires samples to be obtained within 24 hours after death, separated and preferably frozen (not fl/ox tube). Haemolysis can also affect the findings.
- Possible to indicate insulin administration but not dosage, timing, etc.

A number of **complimentary techniques** can be used for screening but the most common procedures at my laboratory are:

Immunoassay - for drugs of abuse

GC-NPD – for basic drugs

GC-FID – for alcohol, solvents and specific drugs

HPLC-DAD – for basic, acidic and neutral drugs

LC-MS-MS – for basic, acidic and neutral drugs, opiates (morphine/heroin)

GC-MS – for basic and specific drugs

It is imperative that a **secondary method of identification** is used for absolute identification (e.g. GC-MS and LC-MS involves both a retention parameter and a mass-spectrum parameter). Some assays do not possess this and therefore are of limited use in forensic cases.

Following initial identification/confirmation, drugs are quantified using the most appropriate technique. e.g. at my laboratory:

HPLC-DAD e.g. antidepressants, cocaine, zopiclone, β -blockers, methadone, benzodiazepines, many other compounds

LC-MS-MS e.g. midazolam, fentanyl, buprenorphine, morphine (inc. glucuronide metabolites), many other compounds

GC-MS e.g. BHB, GHB

UV Spectrophotometry e.g. carboxyhaemoglobin (COHb)

GC-FID e.g. ethylene glycol, valproate, alcohols

With every quantification a set of (~4-5) calibration standards are extracted along with the test sample (in duplicate) and a **quality control** (QC) standard. Results will only be accepted if the QC result is ideally within 10% of the expected spiked value. Participation in external quality assurance schemes is also a necessity for laboratories.

The toxicology report presents the findings of the analysis and if required, interpretative comments. Exact composition varies between laboratories but should contain the following;

- Case details (name of deceased, DOB, reference numbers, etc)
- Specimen details (type, containers, chain of custody, ref numbers, etc)
- Case background and any specific requests
- Clear and full presentation of results (analysis performed with coverage)
- Results per sample (e.g. which exact blood sample was analysed)
- Interpretative comments
- Signed by reporting scientist
- Specimen storage (Human Tissue Act)

Additional recommendations;

References used for interpretation, list of analysts.

SPECIFIC ASPECTS

RESULTS

Units should be obvious and consistent (mass units not molar units): e.g. $\mu\text{g/L}$, mg/L (drugs), mg/dL , $\text{mg}\%$, mg/100mL (ethanol)

Morphine/Heroin: the equipment and methodology is available to determine morphine, its specific glucuronide metabolites and markers of heroin use (e.g. 6-MAM, noscapine and papaverine). These can be useful for interpretation (e.g. source and timing).

INTERPRETATION

The toxicologist should be keeping up with published work and coupled with case experience can provide relevant comments based on the laboratory findings.

The extent and content varies between organisations and individuals but the wording should be clear and appropriate to the results and the initial request.

PROBLEMS AND PITFALLS OF INTERPRETATION

- **No fatal range for all drugs, some overlap with therapeutic range**
- **Drug tolerance (e.g. alcohol, opiates/opioids)**
- **Pharmacogenomics (i.e. fast or slow metabolisers may result in differential “baseline” levels)**
- **Drug stability (e.g. cocaine, some benzos, phenothiazines, diltiazem)**
- **Post-mortem production (e.g. alcohol, GHB)**
- **Post-mortem redistribution (sample collection important)**

Note: tablet counts are not possible based on post-mortem blood findings, calculation is theoretical (V_d) and PM redist. affects concentrations.

WE CAN WORK TOGETHER!

TOXICOLOGIST REQUIRES:

As much initial information as possible regarding the case circumstances

Appropriate specimens, containers, storage and transport

PATHOLOGIST SHOULD EXPECT:

Appropriate, comprehensive, efficient and flexible analysis

Appropriate interpretation – bear in mind that rigid “ranges” do not exist