Appendix D
of the GTFCh Guideline for Quality Control in Forensic Toxicological Analyses

Recommendations for sampling postmortem specimens for forensic toxicological analyses and special aspects of a postmortem toxicology investigation (final part in process)

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1 Introduction and definitions

The purpose of a toxicology investigation on postmortem specimens is to examine whether alcohol, legal or illegal drugs or other substances are directly or indirectly considered as being the cause of death, or whether they have caused incapacity to act or have drugged a victim to incapacitation. For this purpose, collection of suitable and representative samples is an essential requirement.

Definition of terms:
● Specimen: sample material including container
● Sampling: appropriate collection and storage of specimens

Sampling includes:
● selection of sample material suitable for analysis
● sampling at the correct point of time
● sufficient quantity
● suitable sampling technique
● adequate container
● unique labeling
• appropriate storage
• packaging, transport or handing over of sample(s) with a request form
• confirmation of receipt in the laboratory, intermediate storage until analysis is performed
• storage mode and time of remaining material in storage
• disposal of sample(s)
• complete documentation of all individual steps in the procedure (chain of custody)

Forensic pathologists are responsible for the adequate and correct sampling of biological material.

2 Sampling of material for the toxicology investigation during autopsy

The sampling of material for a postmortem toxicology investigation depends on the individual case. As a rule, it is much more complex and challenging than for living persons. Under certain circumstances (e.g. in exsanguination, advanced putrefaction, severe burning), alternative specimens must be collected (e.g. muscle tissue instead of blood). Sampling should be performed such that specimens provide a representative part of the whole. All specimens taken must properly be listed.

2.1 Material for the toxicology investigation

Specimens which can be collected prior to or during all autopsies or cases in which the cause of death remains uncertain or special problems have to be addressed are summarized in Table 1. The recommendations are based on the minimum requirements for sampling postmortem material for a toxicology investigation according to the Guidelines of the German Society of Forensic Medicine "Forensic-medical autopsy“ (AWMF guideline register Nr. 054/001) [1],
Tab. 1: Recommendations for sampling prior to or during all autopsies or autopsies where the cause of death remains uncertain or special problems have to be addressed (see also Tab. 2) [1-4]

<table>
<thead>
<tr>
<th>All autopsies</th>
<th>Additional specimens in cases in which the cause of death remains uncertain</th>
<th>Special problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimens that can be collected <strong>prior to</strong> autopsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood from the vena femoralis</td>
<td>scalp hair, alternatively body hair</td>
<td>Vitreous humor</td>
</tr>
<tr>
<td>alternatively: a sample from the vena subclavia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomitus from the scene</td>
<td></td>
<td>Liquor cerebrospinalis</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td>Finger and toe nails</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin and subcutaneous tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smear tests from the skin and mucosa</td>
</tr>
<tr>
<td>Specimens that should be collected <strong>immediately after</strong> opening of the thoracic and abdominal cavities or after organ removal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart blood</td>
<td>Bile</td>
<td>Muscle tissue</td>
</tr>
<tr>
<td>Gastric contents</td>
<td>Liver</td>
<td>Fatty tissue</td>
</tr>
<tr>
<td>Lungs</td>
<td>Contents of the small and large intestines</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Pericardial fluid</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>Fluid from the thoracic cavity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bones, bone marrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entomological species</td>
</tr>
</tbody>
</table>

If a drug-related case may have had a significant survival time of many hours or several days prior to death, securing of hospital admission specimens by the investigating authorities is recommended. Collection of drug paraphernalia, remnants of drinks or tablets, containers of common household chemicals, and further potential non-biological evidence may provide valuable information in cases of intoxication by unknown substances [2]. In cases of poisoning with gaseous and volatile substances, collection of air samples or samples from the suspected source at the scene of the incident or crime can be useful [4].

2.2 Quantities and special considerations

*Selection of specimens and quantity depend on the circumstances, their availability and the background information. As a precautionary measure, various and numerous specimens should be collected. Samples for which a sufficient data basis for the interpretation of the results is available should generally be preferred for toxicological investigations (Tab. 2).*
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Amount</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood from the vena femoralis alternatively: a sample from the vena subclavia</td>
<td>10-20 mL</td>
<td>For quantitative analyses</td>
</tr>
<tr>
<td>Heart blood</td>
<td>50 mL or total amount*</td>
<td>For screening and general unknown analyses</td>
</tr>
<tr>
<td>Gastric contents</td>
<td>50 mL or total amount</td>
<td>Determination of the total amount is essential; in case of nonhomogenous contents, the total amount should be collected. Tablets, plant components etc. should be collected separately</td>
</tr>
<tr>
<td>Urine</td>
<td>50 mL or total amount</td>
<td>For screening and general unknown analyses, immunoassays; intensive biotransformation can make detection of the parent compound difficult</td>
</tr>
<tr>
<td>Organs (brain, liver, lungs, kidneys, muscle, fatty tissue)</td>
<td>50 g</td>
<td>Large data base for concentrations in liver tissue, lung and brain samples in cases of gaseous and volatile pollutants; few data for concentrations in brain, kidneys and fatty tissue concerning lipophilic substances and anesthetic complications; specimens from kidneys, right and left ventricle in cases of cardiac glycoside poisoning</td>
</tr>
<tr>
<td>Bile</td>
<td>Total amount</td>
<td>Few comparative data, high concentrations for many substances</td>
</tr>
<tr>
<td>Scalp, body hair</td>
<td>A pencil-thick tuft</td>
<td>Retrospective information of chronic or previous use or exposure to drugs, medication or metals. Few data are available for nails or comparing the drug concentration of scalp to that of body hair</td>
</tr>
<tr>
<td>Finger and toe nails</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous humour</td>
<td>Total amount</td>
<td>Detection of e.g. alcohol, heart glycosides, cocaine; diagnosis of diabetes. Few data are available for comparison of vitreous humour to blood concentrations.</td>
</tr>
<tr>
<td>Pericardial fluid</td>
<td>50 mL or total amount</td>
<td>For immunochemical analysis instead of urine</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>Total amount</td>
<td></td>
</tr>
<tr>
<td>Skin and subcutaneous tissue</td>
<td>ca. 2 x 2 x 1 cm*</td>
<td>In cases of subcutaneous injections (e.g. insulin) and percutaneous uptake of a poison, collection of a random specimen from a similar site to act as a control</td>
</tr>
<tr>
<td>Smear tests from skin and mucosa</td>
<td></td>
<td>Clarification of the mode of poisoning</td>
</tr>
<tr>
<td>Contents of large and small intestine</td>
<td>Fractionated where necessary</td>
<td>Metal, plant or mushroom poisoning; suspicion of rectal application</td>
</tr>
<tr>
<td>Fluid from the chest cavity</td>
<td>50 mL</td>
<td>In case of putrefaction</td>
</tr>
<tr>
<td>Bones, bone marrow</td>
<td>ca. 3-5 cm long pieces, &gt; 1 g</td>
<td>In case of advanced putrefaction</td>
</tr>
<tr>
<td>Maggots and other entomological species</td>
<td></td>
<td>In case of putrefaction or advanced decomposition of corpse</td>
</tr>
</tbody>
</table>

* Total amount: maximum amount that can be removed or is still present

Regarding quantities, there are various recommendations in literature [1,2,4]. Quantities listed in Table 2 should be considered as established quantities or correspond to own experience. The specimen's quantity has to be chosen so as to:

- all required analyses can be performed
enough material remains for complementary investigations or repeated examination.

2.3 Sampling techniques

Every specimen must be taken with either a disposable or a clean, dry device. For the removal of body fluids, pipettes with a large diameter or syringes with needles of suitable width and length can be used. For more viscous samples, spoons or ladles; for smears, swabs; for tissue, scalpels, knives or scissors; for gasses, so-called “gas mice” or gas-tight syringes can be used [2, 4].

Preservation of specimens:
Optimally, one blood sample should be collected with a final concentration of 1-5% sodium fluoride, and one in parallel without. For determination of alcohol in vitreous / aqueous humor, addition of sodium fluoride is also recommended. All other samples should be collected without additives.

Blood:
Blood from the femoral vein, or other peripheral veins after preparation of the veins via puncture or incision; where necessary separate sampling of blood from left and right veins; removal of heart blood after opening of the pericardial sack via puncture or incision of the cardiac cavities.

Urine:
Via puncture or directly from the bladder with unhindered view after opening of the abdominal cavity.

Gall bladder fluid:
By squeezing contents into a container; aspiration with a needle after opening the abdominal cavity is seldom effective due to the normally highly viscous contents.

Cerebrospinal fluid:
Suboccipital puncture, or – less recommended – aspiration from the brain's ventricle system after removal of the skull, or via lumbar puncture.

Vitreous humor:
Via a syringe with a fine needle after puncturing the anterior eye cavity, thereafter replacing the fluid removed by an appropriate amount of saline.

Gastric contents:
After opening the abdominal cavity, the stomach should be tied off and then removed, subsequently emptying the contents into a container and documenting the total amount.
Suspicious items such as tablet remnants and herbal matter etc. should be isolated, dried (e.g. on cellulose tissue) and stored separately. If the contents are nonhomogeneous, then preferably the whole stomach contents should be collected.

**Tissue specimens:**
Sampling in separate containers. For cases of poisoning where gaseous or volatile substances are involved, samples of brain, lungs and blood must be collected immediately using gas-tight containers, and if possible, tarred, cooled glass containers. In the presence of penetrating substances or putrefaction, separate removal of portions of the liver near to and far away from the stomach should be done.

**Smears:**
Wiping off of suspicious parts of the skin or mucosa with a cotton wool pad or another suitable adsorbent; in cases of drug death, another area of the skin covered by clothes is wiped off. The adsorbent can be moistened with methanol or another suitable solvent if needed.

**Hair samples:**
Removal is performed preferably from the posterior vertex region of the scalp, except those areas which have come into contact with blood, vomit or putrefaction fluid. A tuft of hair roughly the thickness of a pencil is firmly tied together, and cut off as close as possible to the scalp under light tension. The stubble of hair remaining at the collection site should be noted. Moist hair must be dried. Body hair is removed with a disposable razor or scalpel. Recommendations concerning the correct sampling of hair are made by Tiess [4].

**Bone specimens:**
A piece of cancellous bone (3-5 cm) for example from a vertebra, and a ca. 3-5 cm piece from the femur.

**Entomological specimens:**
As maggots excrete drugs they have taken up very soon after removal from their source of food, they should be briefly washed and frozen immediately after their capture.

**Collection of evidence from the scene:**
Remnants of drinks, fluids or other suspicious materials should be transferred to unbreakable, leak-proof containers and should be packed separately from each other, including the original container itself. All solid matter or containers should be packed separately so as to avoid risk of injury. Gases or vapours can be sampled with a “gas mouse”, or, in the case of an analysis being able to be performed quickly, with a gas-tight syringe. Alternatively, the gas can be transferred from the syringe into a headspace vial [2].
2.4 Containers, labeling, documentation

All containers should not exceed a fill level of 80%. In the case of using headspace vials directly for subsequent investigation, the vapor space above the sample should account for ca. 90-95% of the vial’s volume.

All containers should be disposable, unbreakable and leak-proof. Glass is inert and free of plasticizers, but not unbreakable. Thus, glass tubes should reside in a suitable storage rack and be leak-proof regarding storage and shipment. For sealing the tubes, suitable closures should be used, preferable with Teflon inserts. If volatile or gaseous substances are involved, glass containers are necessary for sampling. For body fluids, disposable tubes made of a suitable plastic material such as Nalgene® can be used. Many commercially available containers with very low amounts of plasticizers made of polycarbonate, polyethylene or polypropylene are suitable for the collection of tissue specimens.

Specimen containers should be labeled with at least:

- The number of the autopsy, or another identification number
- The surname and Christian name of the deceased or another personalized name
- The specimens’ type
- The date of the sampling

If a specimen involves several containers, they should be numbered. All samples, with the exception of hair samples and a sample of femoral vein blood should be combined into one unit and packaged. A packaged unit should, with the exception of the details of the sample materials, contain the same details as the individual specimen containers.

The document accompanying specimens should contain at least the following information [3]:

- The name of the post mortem examiner
- The name of his/her assistant
- Autopsy number, Christian name and surname of the deceased or personalized name
- Date of sampling
- Type and source of sample, quantity (estimated), additives (where used)
- Particular details relating to the sample (e.g. special health risks due to contagious diseases or dangerous chemicals, or details relating to the degree of autolysis)
- Name and signature of the person responsible for checking specimens for completeness after the autopsy has been performed.
- Date and time of transport or handing over of the specimens to the forensic-toxicological laboratory.
2.5 Storage, transport, handing over and disposal of specimens

During their removal and packaging or before storage, the samples should not be left unsupervised, and must be locked away for safe keeping. Only authorised personnel can be entrusted with the handling and processing of specimens.

Before processing, the samples combined into one package should be stored at a temperature of at least –18°C. Hair samples should be stored at room temperature, and a sample of femoral vein blood at 4°C.

If it is necessary to transport the samples, then the cold chain and the chain of custody must be maintained according to the safety regulations and requirements.

On their arrival at the laboratory, specimens should be checked for completeness, intactness and suitability for analysis. Their receipt should be recorded and counter-signed. Annotations concerning any discrepancies must be kept in the laboratory documentation. Every package should be given an identification number. Until their processing and completion of the investigations, the samples must be stored so as to avoid contamination and any changes of the analyte(s) in the material being investigated. If an analysis gives rise to other analyses which are not within the scope of forensic-toxicological investigations, then this should be discussed with the postmortem examiner / pathologist handling the case.

To be able to precisely and economically specify the necessary analyses and to afford sound interpretation of the results, the following information should be made available [3]:

- Request for analysis in writing or as an electronic version
- Name, address and telephone number of the contracting authority
- Autopsy number, or an other internal identification number
- Christian name and surname of the deceased or another unique identifier
- Date of birth of the deceased
- Autopsy report
- Results of the investigation
- Report of the emergency physician
- Medical report or details concerning medication
- Source from which specimens were collected
- Date of specimen collection
- Addition of additives
- Amount of sample
- Risk involved in handling the specimen
- Correct labeling of the sample
- Documentation of the chain of custody
- Specified time frame for processing the specimens.

In the course of the investigation, all portions of the specimens taken for analysis must be documented according to purpose and amount. In the laboratory, a comprehensible record of the investigations should be made, in which the names of the laboratory personnel involved are obvious. During analyses, it must be ensured that the least possible change(s) of the analyte(s) take place. After expiry of the custody period set down in the administrative regulations or after expiry of the time span as arranged with the contracting authority, specimens remaining after investigations are complete can be disposed of. The disposal of the samples must be recorded.

3 References


4 Entry into force

This appendix in its present form was passed by resolution of the executive committee of the GTFCh on the 5th of June 2004 and took effect on the day of its publication in Toxichem + Krimtech.