TIAFT – The International Association of Forensic Toxicologists

Committee of Systematic Toxicological Analysis

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Laboratory Guidelines

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PREAMBLE

Toxicological analysis involves the detection, identification and quantification of toxicologically relevant substances and the interpretation of the results.

In order to obtain reliable results, standards of quality must be applied. The following laboratory guidelines are intended to serve as a basis on which adequate working practices and methodologies can be developed. For some specific applications, national or international regulations already in existence have to be taken into account.

The guidelines apply to the analysis of active constituents or metabolites of pharmaceuticals, addictive drugs and to other toxicologically relevant substances (e.g. alcohol, metals, pesticides etc.) in the broadest sense (mainly in biological samples, [human] body fluids, tissues etc.), including cases of criminal and civil legal relevance, for example:

- *detection of poisons and their relevance in determining causes of deaths*
- *analysis of pharmaceuticals and/or addictive drugs that may impair human behaviour (Human Performance Toxicology/ Workplace Toxicology)*
- *qualitative and/or quantitative analysis of addictive drugs in biological material or other forensic specimens*
- *misuse of substances in relation to sports activities (doping)*
- *environmental toxicological analysis.*

In addition to the qualitative and quantitative analyses as such, interpretation of analytical results is an integral part of toxicological analysis.

These guidelines may also be applied to the toxicological analyses performed for diagnostic and therapeutic purposes (such as in clinical toxicology, transplantation surgery, monitoring or treatment /rehabilitation of drug addicts).

1. LABORATORY AND PERSONNEL

1.1 LABORATORY

Laboratory facilities for toxicological analysis should meet an acceptable standard. Access to the laboratory should be limited to authorised persons.

The laboratory equipment must allow work to an acceptable scientific standard. Laboratory facilities and procedures must allow for the safe handling of potentially infectious and/or toxic biological samples, and prohibit access to specimens by unauthorised persons.

Laboratory procedures must allow satisfactory detection, identification and quantification of individual substances (no groups). At present, acceptable techniques/instrumentation include TLC (thin layer chromatography), GC (gas chromatography), HPLC high performance liquid chromatography), mass spectrometry (MS), spectrophotometric methods (e.g. UV/VIS, IR and atomic absorption) and immunoanalysis (e.g. RIA, EMIT, FPIA etc.). Insufficient technical facilities must not necessarily lower the reliability of the results if any weaknesses are clearly stated in the report (see below), but they will certainly narrow the scope and performance of the analytical process (with respect to analytes detectable, detection limits, meaning of quantitation steps, number of samples to put through, etc.).

1.2. PERSONNEL

The toxicology laboratory must be directed by an appropriately qualified person, preferably with a Ph.D. or comparable university degree in one of the natural sciences, plus additional training and experience. Any member of technical staff must have a professional education adequate to the special responsibilities within the laboratory.

The director must:

- ensure that the laboratory personnel are adequately trained and experienced to conduct the work of the laboratory, and
- maintain the competency of laboratory personnel by monitoring their work performance and verifying their skills, including their ability to act as expert witnesses for the purposes of giving evidence.

2. SAMPLES AND RECEIVING

The proper selection, collection and submission of biological and other samples for toxicological analysis are of paramount importance for the production of accurate and meaningful results as well as for their subsequent interpretation in the adjudication of forensic cases. The director should develop and provide detailed guidelines and instructions to all agencies or parties the laboratory serves. These instructions should state the types and minimum amounts of specimens needed to accomplish the requisite analyses and subsequent interpretations. Whenever possible, the amount of specimen collected should be sufficient to ensure that enough remains for subsequent re-analysis, if required by another party. Instructions should include specific requirements for the type and size of specimen containers and, if appropriate, the type and amount of preservative to be added to biological fluids. Instructions for labelling individual specimen containers, acceptable conditions for packing and transportation should be stated. Submitting agencies should also be instructed how to clearly label (by short

statements "infectious", explained in the accompanying forms) all specimens from living subjects or decedents who may carry a highly infectious disease such as tuberculosis, hepatitis or human immunodeficiency virus (HIV).

Specimens received by the laboratory must be adequately identified and stored in a secure manner such that the integrity of the specimens is safeguarded. Where necessary, acceptable chain of custody procedures should be followed when specimens are transferred from one location to another. Laboratory procedures should minimise any possibility of specimen misidentification or contamination. All specimens must be stored in a secure manner at an appropriate temperature and protected from light during storage. After the initial analysis, residual or duplicate specimens must be stored under appropriate conditions for a sufficient length of time (depending on the analytes, the type of specimen and the purpose of the analysis) to allow for re-analysis, if required. This time should allow for legal process and take into account any regulations which state a minimum period of storage.

3. PRACTICAL WORK IN THE LABORATORY

3.1 ASSURANCE OF SPECIMEN IDENTITY

All aliquots and extracts must be adequately labelled to ensure the integrity of the analytical results. Where necessary, the path of the specimen through the laboratory must be documented by the chain of custody form.

3.2 METHODS

Clear, written instructions must exist for all methods and procedures used in the laboratory (a standard operating procedures manual). Methods should contain sufficient information, such that qualified personnel can follow them after a brief period of instruction. The methods and procedures must be properly validated. All procedures have to be approved by the director of the toxicology laboratory. Any changes in the method or procedure must be clearly documented, stating the reasons for the changes. All changes must be approved by the director of the laboratory or other authorised senior staff.

3.3 ANALYSIS

3.3.1. DETECTION

In qualitative analysis, the first aim is to detect substances of toxicological relevance. Depending on the reason for analysis, different analytical strategies have to be pursued.

If the toxicological analysis is intended to detect a single poison, or a group of poisons, specifically designed analytical procedures will be applied (directed toxicological analysis). Whenever possible, at least two different methods should be applied, each of which uses a different physical or chemical principle to allow for unambiguous detection and confirmation of a substance.

If the analysis is required to detect or exclude a wide range of poisons without specific direction ("general unknown"), the complex analytical strategy of Systematic Toxicological Analysis (STA) is adequate. Its aim is to detect all substances of toxicological relevance, and in positive cases to identify them unambiguously by excluding all other substances except one. To this end, a number of analytical procedures should be run in parallel or in sequence and based on a multitude of analytical principles. (Acceptable techniques /instrumentation will be

outlined in an Appendix.) It should be noted that the above principle usually requires more than running a 'screening' test followed by a 'confirmation' test.

The results from each test are to be checked against appropriate data bases and authentic standards to see which substances would qualify for the response observed, and then the results from all tests should be compared to see how many substances remain that are consistent with the analytical responses. In the end, the results should lead to one candidate.

In order to characterize the degree of certainty of the identification, the methods eventually used to draw conclusions should be stated in the report (see also chapters 4. and 5.). In the event that a separate confirmation test cannot be performed (if only a single analytical procedure is available, or not enough specimen remaining), this must be stated in the report, as it diminishes the certainty of identification.

3.3.2. QUANTITATION

Quantitative analysis should normally only be undertaken where a meaningful interpretation can be expected. Quantitation should ideally be performed on an aliquot of the sample other than that used for screening and/or qualitative analysis.

3.3.2.1. CALIBRATION

Wherever possible, internal standard procedures should be employed, since their use minimises errors due to adsorption onto surfaces, losses during extraction, losses during solvent evaporation, losses during derivatisation, and irreproducibility due to transfer and injection techniques.

Internal standards should ideally be a homologue of the analyte (the drug or other relevant compound being analysed). If this is not possible, a compound possessing similar physicochemical properties to the analyte should be selected.

The chromatographical properties of the internal standard should be such that it elutes close to the analyte whilst remaining completely resolved from any substance which might be present. Wherever possible, the internal standard should be prepared in aqueous solution and mixed thoroughly with the sample prior to the analysis. Calibration graphs should be prepared from standards in the same type of matrix as the sample, and standards must be analysed using the same extraction procedure as the analyte. The calibration graph should be constructed from detector response ratio against analyte concentration for internal standard procedures (whereas a graph detector response against analyte concentration had to be used if no internal standard is applied). If a full calibration curve is not prepared, quantitative standards should be analysed which bracket the concentration of the analyte present.

Results must be expressed unambiguously, and SI mass concentration units should preferably be used.

3.3.2.2. VALIDATION

All methods must be validated using the same primary matrix which will be used routinely in the assay (e.g. blood, serum, organ tissue), to which known amounts of drugs have been added and run through the whole analytical procedure.

(Nevertheless, the criteria of this validation will also depend on the purpose of the analysis. This can mean e.g., that a very low detection limit of a method might not be of real relevance for the investigation of a suspected cause of death.)

Those criteria to be validated are:

- accuracy, precision, absolute recovery (checked at different concentrations), calibration range, selectivity, detection respectively quantitation limit and possibly robustness as well as time and cost for the analysis.

Reagents must be checked by quality assurance procedures. Duplicate analyses should be performed whenever possible. Chromatographic methods of quantification are always preferable to the more classical approaches, e.g. to the use of direct ultraviolet spectrophotometry, since chromatography can separate the drug from its metabolites and other interfering compounds.

The range of concentrations for which the method is valid should be large enough to include all of the concentrations likely to be encountered, e.g. therapeutic, toxic and fatal.

4. REVIEW AND DOCUMENTATION OF THE RESULTS

4.1. QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC)

It is recommended that laboratories have their own internal quality control and quality assurance program, but that they also participate in external quality assurance and proficiency testing programs whenever possible. Depending on the type of analyses (general unknowns, special qualitative analyses, quantitations), method-oriented and/or substance-oriented quality control is needed.

4.2. DOCUMENTATION OF THE RESULTS

Results of all analyses must be fully documented. This written record should include all information necessary to identify the case and its source (together with the additional information about the characteristic circumstances of the case), list the specimens analysed, the substances or groups of substances searched for, should contain all test results and the methods used (explicitly or coded), and must bear the signature of the individual who takes responsibility for its contents. This information should be easily retrievable.

4.3. REVIEW OF THE RESULTS

Before results are reported, each batch of analytical data shall be reviewed by scientific personnel who are experienced with the analytical protocols used in the laboratory. At a minimum, this review should include: chain of custody documentation, validity of analytical data (eg. shape and signal-to-noise ratio of chromatographic peak), calculations and all quality control data. The review should be documented within the analytical record.

5. REPORT

A written report is prepared for the party requesting the analysis. The extent of this report depends on the request. For example, a report for a court file may need to be broader than for a negative drug test in drug abuse monitoring.
The report should contain the specimens analysed, the analytes (substances or groups of substances) searched for. The methods used for the testing should normally be stated by type and include a clarifying statement if results are

less reliable than normal (for example, if no highly informative identification method was included or if a confirmation test could not be performed for reasons already outlined under 3.3.1.). The final results have to be clearly stated and characterized by the corresponding degree of certainty. The report is concluded by the interpretation of the meaning of the results for the ordered purpose of the analysis. Because the results are confidential, every precaution should be exercised to ensure that only a properly authorised person receives the information (especially when it is transmitted by telephone, computer or fax). Each laboratory should formulate its own policy for the retention and release of information.

SUPPLEMENT

3.3.1. QUALITATIVE ANALYSIS

In qualitative analysis, the first aim is to detect all substances of toxicological relevance. Then, the next aim is to unambiguously identify the substances found in the detection stage.

Since the outcomes of these analyses can have substantial legal and/or social consequences, all approaches and procedures should be scientifically undisputable and legally defensible.

In all cases, the relevant properties of the analytical procedures used (e.g. selectivity, sensitivity, robustness, reproducibility, etc.) have to be adequately ensured and considered and documented in the analytical report.

3.3.1.1. Detection

Depending on the reason for analysis, different analytical strategies may be pursued. If the toxicological analysis is intended to detect a single substance or a group of substances, e.g. in workplace testing, specifically designed analytical procedures can be applied (directed analysis).

If the analysis is required to detect or exclude a wide range of (potentially toxic) substances without specific direction (undirected analysis or 'general unknown'), the comprehensive strategy of Systematic Toxicological Analysis (STA) is required.

Its aim is to detect all substances of (actual) toxicological relevance. To this end, a number of analytical procedures should be run in parallel or in sequence, representing a multitude of analytical principles. Prior to the systematic analytical approach, thorough consideration should enable to reasonably confine the scope of the detection stage to compounds relevant to the actual problem. Experience with similar tasks may also be considered when possible.

Therefore criteria to define the group of relevant compounds for a given area of interest are desirable. The various areas of interest (such as forensic and clinical toxicology, workplace testing, drugs of abuse testing, drugs and driving, doping analysis, environmental analysis, residue analysis) represent analytical challenges of their own, that should be taken into consideration when embarking on the systematic analytical approach.

3.3.1.2. Identification

When the detection procedures indicate the possible presence of one or more toxicologically relevant compounds, the latter have to be unambiguously identified.

This can be done by comparing the signals (results) of the various tests applied in the detection and/or identification process from the unknown sample with data from authentic reference standards analysed under the same actual conditions and/or (Iess reliably due to additional variables) with data on reference compounds stored in appropriate data bases on relevant substances.

The ultimate aim of the identification process is that for a given unknown substance only one suitable candidate is found (because all measured signals of the unknown and the reference candidate match adequately) and that all other relevant substances can be excluded (because one or more signals do not match). Experience has learned that a single analytical method, even when it is based on a highly informative principle, is not always sufficient to reach unambiguous identification. The large number of substances, sometimes widely different, sometimes with

very close structural resemblance, make it hardly possible to really fulfil the exclusion criterion. Therefore, proper identification requires as a rule two, if not more analytical methods (their number depending on their information gain), to exclude all possible candidates except one.

Ideally, the analytical signals for the unknown(s) should be compared with those of authentic reference standards run in parallel with the case sample. This is more secure than comparisons with literature data or such stored in data bases, because the data may be influenced by the actual analytical conditions. However, keeping up an adequate collection of reference substances is easiest for certain areas in which the number of relevant substances is small (e.g. workplace testing). When the number of compounds of interest is very large (e.g. in forensic and clinical toxicology, control of drugs and driving), it can become very difficult for single laboratories to set up and maintain adequate supplies of all reference substances (and of their metabolites). In these instances, the use of reliable, interlaboratory data bases might be the only feasible solution. The data collection must then contain not only the toxicologically relevant substances, but also metabolites, related substances (including isomers, sometimes enantiomers), endogenous substances, and the like. In addition to the data themselves, the interlaboratory reproducibilities of the analytical techniques must be available and have to be included in the evaluation of the compared results and in the conclusions.

In recent years, many analytical toxicologists have come to use the term 'confirmation' of a first analytical 'screening' step as a substitute for 'identification'. When this relates to cases in which the results from the detection or screening phase lead to the presumption that a certain substance is present, and in the confirmatory stage one or more signals from the unknown are matching those of the presumed candidate, the presumption is considered 'confirmed'. However it should be realized, that such an approach does not necessarily provide unambiguous identification: it will always depend on the existence of similar analytical signal patterns of other compounds and on the actually provided information capacity, whether another substance cannot be distinguished from the presumed one. Thus, it has always carefully considered whether the exclusion criterion mentioned above is fulfilled.

In order to enable others to estimate the degree of certainty of the result of a qualitative analysis, the methods applied to draw conclusions should be stated in the report (see also chapters 4. and 5.), eventually together with their appropriate properties.

Special circumstances, such as limited specimen supply, unavailable or improperly functioning detection and/or identification techniques, unexpected interferences, etc., must be mentioned if occurring in the report as well.