GUIDELINES FOR LABORATORIES ANALYSING SUBSTANCES OF ABUSE FOR FORENSIC PURPOSES

Study Project of the Group of Forensic Toxicologists (GFT) at the Ministry of Health

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INTRODUCTION TO THE GUIDELINES

These Guidelines were prepared by the Commission of Forensic Toxicologists at the Ministry of Health, in the year 2000.

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This (1st) revised version of the Guidelines is a follow-up of the initial version of the same Guidelines, which was issued in March 2001.

The proposals contained in the Guidelines were discussed by the GFT (Group of Forensic Toxicologists of the Società Italiana di Medicina Legale e delle Assicurazioni) during a consensus forum held under the aegis of the Ministry of Health.

It was decided to prepare this revised version in order to better explain the quality-related concepts that should be kept in mind by laboratories when analysing substances of abuse for forensic purposes. In addition, a few purely “forensic toxicological” aspects contained in the previous version were improved.

This revised version of the Guidelines was prepared after:

- Developing and improving the concepts and purposes of the “Guidelines” that had already been formulated by the GFT in the previous version;
- interpreting the norm UNI EN ISO 9000:2000 “Quality management systems (Basic notions and terminology)”;
- interpreting the norm UNI EN ISO 9001:2000 “Quality management systems (Prerequisites)”.

The norm UNI CEI EN ISO/IEC 17025 “General requirements regarding the competencies of laboratories carrying out tests and calibrations” was also taken into consideration; although this norm is considered too cumbersome for the time being, it may be required in certain specific diagnostic sectors in the future (Law 376/2000). The norm contains various suggestions that strengthen the application of the two above-mentioned ISO norms, and its possible future application would in no way be compromised or impeded by them.

When preparing the Guidelines, the GFT Commission at the Ministry of Health decided to divide it into 9 sections:

- Purpose and application of the Guidelines
- Terms and definitions.
- Personnel
- Procedures (documented procedures - Standard Operational Procedures)
- Requirements for carrying out analyses
- Acceptance, conservation and recording of the samples
- Analytical procedures
- Guaranteeing quality – assurance
- Analytical reports.

THE PURPOSE AND APPLICATION OF THE GUIDELINES

1.1 Purpose

These Guidelines were elaborated and then revised (this is the first revised version) by the Commission of Forensic Toxicologists at the Ministry of Health.

The GFT felt the need to prepare this type of document for the following reasons.
Drug abuse analysis for forensic purposes must be continually improved, both to consolidate the analysis techniques already in use and to acquire new ones, and to identify analytes and biological samples other than those that are traditionally used for analysis.

The results of toxicological analyses of substances of abuse may be used as judicial evidence; therefore, by definition, they must observe certain requirements regarding transparency and uniformity that must be constantlytraceable during the activities of all the laboratories working in this specific sector.

The high quality of the results of the toxicological analyses of substances of abuse is guaranteed by utilising methods and techniques that have been widely experimented, but also by the certainty that such results are produced in efficient and reliable structures.

Finally, there is a strong national demand for uniformity in the treatment, analysis and interpretation of all those elements that produce the results of toxicological analyses of substances of abuse.

Therefore, the aims of these Guidelines can be summarised as follows:

- To provide all the laboratories involved in this specific sector with a universally operational tool that allows those laboratories to perform their work correctly in the field of forensic toxicology;
- To provide suggestions and recommendations on how to deal with each analytical process;
- To ensure the availability and utilisation of the necessary resources;
- To advise the structures on how to issue and handle the results of toxicological analyses of substances of abuse.

1.2 Application

These Guidelines were drafted with the following objectives in mind:

- To disseminate and make applicable the analytical principles that are essential when analysing substances of abuse for forensic purposes, based on the years of experience of the GTF;
- To allow laboratories carrying out forensic analysis of substances of abuse to acquire the requisites of what should become an efficient and reliable structure over time, by adopting a quality control system that was planned and realized taking into account the characteristics of the laboratories themselves.

Therefore, these Guidelines were created in order to provide an element of self-discipline and what is declared to be an essential prerequisite of a quality control system adopted in laboratories carrying out forensic analyses of drugs of abuse. They should become an essential component in any process to obtain an “accreditation of excellence”.

It is therefore hoped that forensic toxicological laboratories will adopt quality control systems that express and verify the internal quality control policy adopted by each of them. These policies should have the following objectives:

- Excellent results and efficient organisation;
- Constant improvement in the quality of the service provided;
- Quality seen as the responsibility of everyone;
- Making the staff responsible for guaranteeing the quality of the work for which each of them is competent;
- Promoting a policy based on quality among all the laboratory staff;
- Constant updating of the policy and its objectives.

1.3 Implementation

These Guidelines must be adopted and implemented by all laboratories carrying out forensic analyses of substances of abuse in biological material, which will hereinafter be referred to as “toxicological analysis laboratories” or “forensic toxicological analysis laboratories”.

The laboratories authorized to perform the above mentioned analyses should therefore observe the principles contained in these Guidelines with respect to organisation and methodology, so as to ensure that the uniform requisites needed to guarantee quality are implemented and can be verified.

Such requisites regard:

- The staff structure, and the identification of the competencies and responsibilities of the staff
- The procedures to be used when acquiring and analysing samples
- The interpretation of the results, also taking into account predetermined cut-off values
2. TERMS AND DEFINITIONS

Accreditation: a voluntary process aimed at continuously improving quality, whereby an independent body evaluates whether an institution or programme satisfies certain predefined requisites.

Confirmatory analysis: a second analysis to be performed using a method that is more specific than the one used for screening, on a different part of the original sample, in order to identify and/or quantify the presence of a substance or its metabolites.

Screening analysis: preliminary analysis that determines the positivity or negativity of a sample with reference to predefined cut-off points.

Forensic toxicological analyses or analyses of drugs of abuse for forensic purposes: for the purposes of these Guidelines, these are analyses that are performed on biological material, in particular on drugs of abuse, for forensic purposes, and which may therefore be used as judicial evidence during the enforcement of current laws.

Batch: a group of samples that are examined in series

Qualitative analysis: a toxicological analysis that only provides a result regarding the presence or absence of an analyte.

Quantitative analysis: a toxicological analysis that is able to identify the level of concentration of the analyte in the examined biological sample.

Chain of custody: documented procedure aimed at reconstructing the course of each sample within the laboratory, to identify its current allocation or possible removal from the laboratory, to identify all the names and dates of the people that have handled the sample from when it was delivered/entered the laboratory up to when it was moved or disposed of, in accordance with the procedures in force (B).

Cut-off or threshold value: defined concentration limit to establish whether a sample is positive or negative.

Blind check: an undeclared control to check that the envisaged procedures are implemented, or a sample containing a predefined or negative analyte concentration in order to evaluate the laboratory’s ability to perform a specific test; the laboratory does not know the concentration.

Non-blind control: a sample containing a predefined quantity of analyte, set up in a biological matrix similar to that of the real samples.

External quality control: external monitoring of the analytical reliability of the laboratory, which is evaluated on the basis of the qualitative-quantitative results obtained from the analysis of a series of blind checks.

Internal quality control: monitoring of the methodologies used by the laboratory, through the analysis of blind and non-blind checks.

CV: coefficient of variation or relative standard deviation, frequently used to measure reproducibility. It is obtained from the percentage ratio of the standard deviation over the average value.

Limit of identification, calculated on the basis of an average white value to which is added a value that is normally three times the standard deviation.

LOQ: limit of quantification, may be calculated by adding to the blank value, the standard deviation multiplied by ten; however, it is preferable to determine the LOQ experimentally as the lowest concentration needed to obtain an acceptable CV.

Standard (Methodological) Operational Procedures: the orderly sequence of actions and events that must be followed when carrying out analyses in standard laboratory conditions.

Documented Procedures: description of interactive activities.

Operational instructions: descriptions of the tasks regarding a single function other than the performance of an analysis.

Negative result: non-identification of the analyte, in accordance with the laboratory’s internal analytical procedures.

Positive result: identification of the analyte in accordance with the laboratory’s internal laboratory procedures.

Quality control system: series of correlated or interacting elements used to establish policies and objectives and to obtain those objectives, in order provide guidance and constant quality control in an organisation.

Process: a series of correlated and interactive activities (e.g.: acceptance, analyses, the production of results, conservation of the samples, etc.) whereby incoming elements (request to carry out analyses) are transformed into outgoing elements (forensic analytical report).

Outcome: results of a process.

Quality assurance: part of the quality control system aimed at ensuring that the requisites for guaranteeing quality are observed.

Traces: semi-quantitative expression used to indicate the presence of an analyte in concentrations that are lower than the lowest calibrator in the analytical system used.
Analytical report: outcome of the analytical procedures carried out in the laboratory, i.e. all the correlated or interactive activities whereby incoming elements are transformed into outgoing elements.

3. PERSONNEL

3.1 Laboratory directors

The directors of forensic toxicological analyses laboratories are responsible for professional, organizational, educational and administrative aspects. The person holding this position must have a suitable science degree and a specialisation in chemical-toxicological diagnosis, with documented proof of completion of a training course, practical experience, up-to-date knowledge and the possible authorship of related written material.

3.2 Laboratory Staff

The staff must have adequate professional training to be able to perform their tasks within the laboratory, and a particular knowledge of the norms relating specifically to forensic toxicological analysis laboratories. The director must ensure that the laboratory staff are adequately qualified, trained, cultured and experienced to carry out their functions. The director should make sure that they continuously attend training and refresher courses, and should keep records of certificates confirming completion of such courses. The director should also ensure staff compliance with the laboratory procedures.

An adequate number of staff members must be assigned to carry out a given number of analyses, and should be capable of following predetermined analytical procedures. The number of analytical procedures should be commensurate with the number of laboratory staff and the equipment used in the laboratory.

At least one person with a suitable degree in science and with adequate experience in toxicological analysis (with documented proof of the completion of training, practical experience, up-to-date knowledge and the authorship of publications) should be present to supervise the work, verify the results of the various tests and report to the director about progress made in the laboratory.

In compliance with a quality management system, this professional figure should receive the necessary training, so as to be able to be delegated by the director to act as the “Representative of the Direction”, and thereby to control that the adequate procedures have been adopted, as well as making suggestions on how to make improvements and satisfy customers.

3.3 Minimum safety rules

The laboratory must take the necessary measures to guarantee the safety of its staff. In particular, adequate instructions should be provided on how to perform analytical procedures that may pose a health risk. The director should ensure the observance and application of enforced norms regarding the prevention and protection of staff health. Strict rules regarding the handling and disposal of at-risk materials should be enforced.

4. PROCEDURES

4.1 General remarks

The forensic toxicological laboratory must collect in a document, descriptions of the criteria to be followed when carrying out all the procedures involved in the production of the end product (the analytical report), i.e. from acceptance of the analyses and the related samples, to the submission of the results. This document must be organised according to the normal procedures used in quality management systems (Quality Manual, documented procedures and instructions regarding laboratory operations); the document should comprise a collection of the Standard Operational Procedures which describe in detail all the operations to be carried out in order to correctly perform all the analyses conducted in the laboratory.
The documents (criteria for performing procedures and Standard Operational Procedures to be used when carrying out analyses) must be clearly identified, complete, up-to-date and always at the disposal of the staff carrying out analyses. It must be prepared by the Laboratory Director and should include the dates of the first and successive drafts; any revised elements must be noted on the revised version.

4.2 Documented procedures
The documented procedures describe each process carried out within the laboratory. Access to, and observance of the documented procedures that are constantly updated and controlled, will guarantee that all the foreseen procedures are carried out in a homogeneous and reproducible manner.

The documented procedures must contain at least the following:

- procedures to define the requisites of the product to be supplied (type of analysis and its purpose) and assistance to the customer;
- procedures to be adopted for the chain of custody;
- procedures regarding the acceptance and identification of the sample;
- procedures on the utilisation and maintenance of the equipment;
- procedures for internal and external quality control;
- methods to be used to transcribe the results and prepare the analytical report;
- procedures for protecting and ensuring the confidentiality of the results and of delicate health-related data;
- procedures regarding monitoring and improvement.

4.3 Standard Operational Procedures
The Standard Operational Procedures describe in detail all the activities to be performed in order to correctly carry out any type of analysis or diagnostic test performed by the laboratory; they contain an analytical method or procedure and establish the order of actions and events in order to ensure that analyses are conducted in standard conditions in use in the laboratory.

4.3.1 Requisites of the Standard Operational Procedures
Each Standard Operational Procedure should include details of:

- The purpose of the analysis
- Principle of the analytical method including any bibliographical references
- Operational details regarding the reagents (composition, preparation, precautions to be taken during use, conservation, characteristics relating to instability or deterioration);
- Preparation of the sample, calibrators and controls;
- The identification, position sequences and procedures for preparing the standards;
- The identification of instruments, their functioning and means for calibrating them;
- Acceptable defined criteria for producing the data;
- Characteristics regarding specificity, sensibility and accuracy.

Each Standard Operational Procedure should include a control of the quality of the analysis system, in order to guarantee that the quality of the data produced is equal to that of the validated method. Each Standard Operational Procedure should foresee the corrective procedures to be adopted if the results of the controls exceed the acceptable limits.

4.3.2 Implementing the Standard Operational Procedures
Each Standard Operational Procedure contains an analytical procedure (see section 7) that is utilized by the laboratory and included in the laboratory’s official collection of documents after it has been accepted and validated by the director. Any external procedure used in the laboratory must meet the official scientific requirements, or, if the method used is the outcome of the research activities of the laboratory itself, by similar requisites that can be traced in the documents recording the developments of the research activities.

Each analytical procedure must be performed in full observance of the Standard Operational Procedures contained in the laboratory’s official collection. Each review of the Standard Operational Procedures must be recorded and the modified and substituted procedures must be kept in an easily accessible file.
5 REQUIREMENTS FOR CARRYING OUT ANALYSES

5.1 Diagnostic purposes

Toxicological analyses for various diagnostic purposes may include tests on different biological samples (such as blood, urine, hair, saliva, sweat) which may be analysed singly or in combinations in order to obtain forensic diagnoses for various cases, including: ascertainment of ability to drive, road accidents, at-risk work duties, weapon licences, compliance with specific competition rules, child assignment, annulment of marriages, international adoptions, etc.

Whenever the laboratory prepares an analytical report containing an evaluation on the “current use of illicit substances of abuse”, such evaluation can only be made following the performance of a blood test.

5.2 Biological samples

The laboratory assigned to carry out toxicological analyses should be able to guarantee that analyses are performed on at least one of the following biological samples: blood, urine, hair.

The minimum quantity of a biological sample deemed necessary to perform toxicological analyses must be clearly indicated in the standard operational procedures, and must be sufficient to allow for a replication of the analysis, to guarantee the number of analytes being analysed, and to meet the qualitative and/or quantitative aims of the test.

The methods used to take, conserve, transport (if applicable) and store each sample must be clearly indicated.

5.3 Controlling the procedures

The laboratory must provide its services and perform its activities in controlled conditions. In order to achieve this, the laboratory may adopt a quality management system for all the activities it performs; this system must in any case comprise all the activities related to forensic types of activities.

The laboratory must declare that such activities are carried out in observance of the “Guidelines” which is understood to be an internal control mechanism.

The quality management system may be limited to screening (and only provide qualitative evaluations), or it may also include the confirmatory analysis and thus also provide quantitative results; when declaring its internal requisites, the laboratory must indicate the field of application of its quality management system.

6. ACCEPTANCE, CONSERVATION AND RECORDING OF THE SAMPLES

6.1 Access to the laboratory

Only authorized persons may have access to the laboratory.

Unauthorized persons must be accompanied and their names noted in a special register.

6.2 Acceptance

Laboratories that also perform toxicological analyses on non biological samples should acquire, handle and stock the biological samples in separate environments in order to avoid contaminating the environment.

The laboratory should document the adoption of appropriate procedures needed to observe the methods foreseen by the laboratory to preserve the characteristics of each sample during all the laboratory procedures that it undergoes, from sampling until it is returned to the client, or is disposed of.

The laboratory should also provide evidence of all the operations performed on the sample, and adopt suitable procedures for all the envisaged phases in order to complete the entire process. These procedures should become an integral part of the documents that are part of the quality management system.

Through these procedures, the laboratories should define the operational method to be observed when carrying the following activities.

6.2.1 Acceptance of the request to perform analyses

- The laboratory should record the following:
  - the identify of the requesting party (private person, entity, forensic doctor delivering the sample, etc.) whose means of identify should be recorded (entities are excluded from this requirement);
  - date of the request;
o name of the persons taking the sample;
o evidence that the compatibility of the sample with the analytical procedures to be performed has been verified;
in the case of urine, if necessary, the laboratory should be able to identify and record the chemical-physical characteristics of the sample (density, temperature, colour and pH);
o circumstantial elements (therapies being undergone, etc.);
o purpose of the analyses;
o clear identification of the sample and its allocation during acceptance;
o evidence that the requesting part is aware of the possible consequences of the results of the requested analysis;
o clear identification of the person accepting the request.

6.2.2 Taking the sample
The laboratory must take the necessary measures to ensure that:
o only authorized persons working for the structure have access to the place in which the sample is taken;
o the person providing the sample leaves any bag and other unnecessary item of clothing (jackets, overcoats, etc.) in a different place;
o the person providing the sample may only be allowed access to the room and receive the material needed to take the sample, after carefully washing and drying his/her hands;
o in the case of a urine sample, that there are no sources or materials in the room that may be utilised to dilute or modify the sample (running water, containers for soap, disinfectants, household cleaning materials, etc.). It should also be possible, if necessary, to directly observe the interior of the room.
o The person authorized to take the sample informs the person providing the sample about how the biological sample will be taken, divided and labelled;
o the material needed to take the sample is supplied in an integral and sealed container and that the sample is labelled in the presence of the sample supplier;
o the sample is identified on the labels of the containers utilised;
o the sample is correctly preserved in order to protect it against adulteration, pollution or dispersion, through the use of suitable and perfectly sealed containers that resist damage during transportation, and thermal shock during the freezing phase;
o the sample can be traced during each phase of the analysis;
o if the samples are transferred to another place other than that where they were taken, that the necessary measures are taken to guarantee the “cold chain” and to impede any manipulation of the samples;
o sufficient quantities of each biological sample should be taken, depending on the aim of the tests, to replicate the analysis, if necessary, and to perform a further confirmatory and/or review analysis when this is requested.

6.3 Conservation, handling, and transfer of the sample
Each laboratory should adopt the appropriate means to correctly conserve the sample.
These should ensure that:
o The laboratory performs the correct procedures to conserve the samples; in particular, it should define and identify the places in which the samples are to be stored, which should be suitable for conserving samples during all the phases of the analyses. As regards samples that must be kept at low temperatures (blood and urine) different refrigerators must be foreseen for short, medium and long-term storage; samples that are to be analysed immediately or within 48 hours should be stored in a refrigerator (approx. +4°C ) while those that will undergo a confirmatory or review analysis should be stored at approximately -20°C;
o The laboratory carries out suitable and documented transversal procedures to be able to immediately trace the sample during each phase of its transfer; such procedures, such as the “Chain of Custody”, must make it possible to:
   ✈ identify the person that handed in the sample;
   ✈ identify the person that accepted the sample;
   ✈ identify where the sample is located at a given time;
   ✈ know the names of all the people that have moved the sample;
   ✈ trace the date of each movement of the sample;
   ✈ identify the person who collected the sample.
Records of the application of these procedures should be kept at least until the sample has been returned or disposed of;
6.4 Control of the monitoring and measurement tools

The laboratory should identify and monitor the “critical” physical sizes, i.e. those that influence the quality of the final outcome of the analytical procedures, in order to correctly conserve the samples and keep them under control through the use of suitable tools.

These operational procedures should be extended to include the auxiliary tools that are “essential” for obtaining the desired level of quality.

Such controls should regard at least the following:

- the temperatures of the refrigerators in which the samples are conserved (as well as the reagents which must be conserved in the conditions indicated by the makers in order to ensure a high quality process) through the use of regularly checked tools (thermometers);
- the volumetric tools utilised both to prepare and treat the samples, through the use of appropriate tools (e.g. scales) that are periodically checked.

7 ANALITICAL PROCEDURES

7.1 General remarks

The Standard Operational Procedures contain the analytical or methodological laboratory procedures that should guarantee the best possible identification and dosage of the analytes, compatibly with the instruments available in the laboratory.

The analytical procedures should aim at directly identifying each analyte and should ensure the minimum possible level of alteration of the single metabolites, when these can also be produced as artefacts in the laboratory.

A description of each analytical procedure adopted by the laboratory and evidence of its validation should be kept. The field of application regarding the matrix and the type of analytical parameter to be determined should be indicated for all the analytical procedures. The following scheme is recommended when preparing the analytical procedures:

- Warnings and precautions
- Scope and field of application
- Bibliographical references
- The principle and the treatment of the sample
- Reagents and standards
- Equipment
- Calculations and data transfer
- Data quality control.

Each procedure must be identified and indicate the number of the revision, the date of the review, any changes that were made, the person carrying out the review, and enumerated pages that are identified as part of the document.

7.2 Screening

Analytical procedures normally involve a first screening phase following by a confirmatory phase, based on different physical chemical principles.

Immunochemical or immunometric techniques (EIA, FPIA, RIA, FU, etc.) may be utilised as screening methods.

The immunochemical screening methods are normally used to provide analytical results regarding presence or absence, with reference to the cut-off value. The selected cut-off values must be those normally accepted in the international scientific community.

When used for forensic purposes, the results of screening obtained using immunochemical methods must be confirmed by using other analytical methods that are more specific and sensitive than the screening method and are based on a different chemical-physical principle.
7.3 Confirmatory analyses

An important scientific principle, which is essential during toxicological analyses carried out for forensic purposes, is to confirm the initial identification of an analyte. It is therefore mandatory to carry out confirmatory analyses when providing a final result, especially if the screening is carried out using an immunochemical method.

Confirmatory techniques should be more specific and sensitive than screening analyses. Generally speaking, it is advisable to utilise gas or liquid chromatography combined with mass spectrometry to confirm an analysis. All the positive results and at least 5% of the negative results of an immunochemical screening must be confirmed.

The analytical result obtained using an immunometric method cannot be confirmed using a second immunometric procedure, even if this is based on different identification principles (level of radioactivity, enzymatic kinetics, fluorescence, agglutination, etc.) and antibodies with different specificities are used. In the same way, closed chromatographic systems that are not able to characterise the molecular of the analyte or which are not specific enough are considered screening systems and cannot be used as confirmatory analyses.

It is advisable to perform the confirmatory analysis on a second part of the biological sample which is different from the one on which the preliminary screening was effected.

The quantitative analysis of an analyte that was previously identified using the screening method (for example, dosage in GC-MS using SIM after the immunoenzymatic dosage) may be valid as a confirmatory analysis. When the quantitative analysis is carried out using mass chromatography followed by mass (HPLC-MS or GC-MS) using selected ion monitoring (SIM), at least three significant ion fragments must be considered for each analyte. When the gas chromatography mass-mass (GC-MS-MS) is used, the quantitative analysis should be performed on the most significant ion in the MS-Ms spectrum.

Gas chromatography analysis after derivatization may be used to confirm the preliminary screening also carried out using gas chromatography on non derivatized analytes. However, the analysis must be carried out on different extracts of the same sample.

7.4 Validation

All analytical procedures must be validated before being utilised. Each laboratory must validate the method chosen to determine the analyte on the basis of the nature of the matrix. The criteria normally utilised to validate the method are:

- The performance of recovery tests on samples without any residuals of the analyte to be determined, with a stronger known concentration of the reference standard containing the same analyte.
- The use of a certified standard of reference.
- The establishment of the number of recovery tests to be carried out, as well as the concentrations to be used and the interval in which the average recovery values should be completed for each level of concentration, and the limits of acceptability of the standard deviation.

A minimum quantity that can be revealed using the confirmatory methods must be determined with reference to the standard blank deviation value. The laboratory may determine the limits of deviation (LOD) by calculating the average blank value and adding to it a value that is normally three times the standard deviation. The coefficient of variation values (CV) or the reproducibility characteristics should be known for each analytical procedure. The limit of quantification (LOQ) may be determined by adding ten times the standard deviation to the blank value; however, it is preferable to determine the limit of quantification (LOQ) experimentally as the lowest concentration needed to routinely obtain an acceptable coefficient of variation.

7.5 Reference Standards, calibrators and analytical quality controls

7.5.1 Reference Standards

The Standards, accompanied by the documented source and date of the preparation, must be suitable for the type of analysis being carried out. The stability and integrity of the reference standards must be preserved during their conservation. If the standards are prepared in a laboratory, the firm producing the reagents must keep records of the method used to prepare them, and of verification of the end product.

The standards of pharmaceutical substances, drugs and metabolites acquired in accordance with the laws in force, have a limited duration. They must be controlled regularly for any degradation due to light, humidity or low temperatures. Standards in a solution may remain stable for shorter periods of time than those in pure, dry or solid substances.
The identify and the degree of purity of the standards, including those in a deuterated form, must be verified using suitable procedures and optimal instruments, above all if they are not adequately certified by the manufacturers. A uniform labelling method must be used for all the standards and reagents. The labels should contain the date of receipt and preparation, the initials of the person who prepared them and their date of expiry.

For the chromatographic methods (GC, HPLC) it is advisable to use internal standards and in particular internal deuterated standards in the case of CG-MS. It is essential to use internal standards for methods based on head space gas chromatography (HS-GC) techniques. The internal standards, to be selected on the basis of appropriate chemical-physical evaluations, must be added to the biological sample before commencing the analytical procedure.

7.5.2 Calibrators

Calibrators are used to determine the curve of calibration, which must comprise a blank and various calibration points. The linearity of the analytical procedure must be evaluated using at least three calibrators with different concentrations. If a sample contains an analyte with a concentration that is greater than the highest calibrator, the analysis must be repeated after diluting the sample.

Analytical procedures and control systems must be utilised that guarantee the identification of any carry-over problems.

7.5.3 Analytical quality controls

Control samples with known concentrations (including negative controls) must be included in each analytical session. It is essential for the controls to have the same matrix of the sample to be analysed. Each laboratory must identify and utilise (and explain the reason for this choice) control samples that establish the limits of acceptability of the session. Non-blind checks, containing a known quantity of standards (pharmaceutical substance(s) / drug(s) / metabolite(s)), must be as similar as possible to the biological samples to be analysed. The concentrations of the analytes contained in the controls (purchased on the market or prepared in the laboratory) must be confirmed. If analyses are carried out on unusual matrices (hair, nails, bone, tissue, etc.) the controls must be prepared with similar matrices, identical matrices ("negative" controls) or with matrices to which analytes have been added ("positive" controls). Normally, an adequate series of controls may be limited to a sample that does not contain the analyte ("negative" control) and to a sample containing the analyte ("positive" control), at a concentration that is suitable for controlling the reliability of the analysis.

Additional controls may be used to evaluate the linearity of the calibration within the established concentration interval. For each batch of single or multiple biological samples, the controls must be carried out using the same parallel procedure adopted for the unknown samples. Each batch of biological samples must include at least 10% of the controls, including a “positive” and a “negative” one. The “control” must produce a result that falls within a predetermined deviation from its average value; otherwise, the test is considered “out of control” and the result for an unknown biological sample is unacceptable.

Laboratories that carry out forensic toxicological analyses should participate in an external quality control programme, in order to constantly monitor the analyses (including the efficiency of the calibration tools) as well as to optimise them, in particular with respect to analytes and biological matrices that are analysed by the laboratory for forensic purposes. Blind checks should be performed (and the reliability of the results verified) both on samples of which the identify and composition are unknown to the analyst, and on all the activities of the laboratory, i.e. acceptance and registration of the sample, analysis of the results of the tested biological samples, verification of observance of the procedures adopted in the laboratory.

8. GUARANTEEING QUALITY – QUALITY ASSURANCE

The directorship of a laboratory carrying out forensic toxicological activities should aim at guaranteeing that the requisites to ensure quality are met: in other words, it should provide real evidence that “quality is guaranteed”.

8.1 Guaranteeing quality

Assurance is particularly important in forensic toxicological analysis since the results may be used as “judicial evidence”.

Commissione GTF presso il Ministero della Salute
The aim of ensuring quality consists in identifying and implementing mechanisms that are able to identify errors, setting up devices to control them and adopting the necessary measures to prevent their reoccurrence; these mechanisms produce a sense of trust in the laboratory’s capacity to meet assurance requirements when carrying out its activities. Assurance covers all the procedures performed within a laboratory, from receipt and acceptance of the biological samples, to the performance and validation of the analyses, up to providing the results. Quality is guaranteed by carrying out assurances in the laboratory.

Assurance implies the use of appropriate documents, and the recording of the laboratory activities, through the use of procedures aimed both at controlling the procedures (documented procedures) and developing the analytical procedures (Standard Operational Procedures).

8.2 Quality assurance

Laboratories that perform forensic toxicological analyses must ensure that part of the duties of the management is to attempt to meet quality requirements and provide real evidence that quality has been guaranteed.

The laboratory must acquire an internal quality system and record its implementation.

The controls should regard at least the following aspects.

- Samples during the phase of acceptance (quantity, quality, etc.);
- The equipment being utilised during the analytical process (tool calibration, calibrators, operational consensus);
- The carrying out of the analysis by including the blind and non-blind internal and external control samples described in section 7.5.3 “Analytical quality controls”;
- The preparation of the results by adopting procedures regarding delivery of the end product.

The results of the controls must be recorded.

9. ANALYTICAL REPORTS

9.1 Requirements for the analytical report

The analytical report should always be prepared in a written form only and is normally delivered to the party that requested the tests or to another person in possession of a written delegation. The analytical report must include, in addition to the date and all the information needed to identify the sample, the method used to carry out the analysis, the cut-off value and, in the case of quantitative analyses, the limit of sensitivity of the method used. To be more specific, the analytical report should contain the following elements:

- title;
- identification of the laboratory: name and address;
- a univocal identification of the document (progressive number), numbered pages that are recognisable as being part of the document, clear evidence of the purpose of the document;
- name and address of the client or request code;
- identification of the method used;
- clear identification and description of the analysed samples, as well as their condition;
- date of receipt and analysis of the samples;
- references to the sampling methods used;
- the results of the test with the units of measurement and the cut-off values;
- names and functions of the persons signing the report (the results should always be signed by the director of the laboratory);
- purposes of the tests;
- if required, a declaration that the results of the evaluation and their interpretation refer to the analysed samples only, as well the level of utilisation and limits of the results;
- there must be a trace, in the analytical report (or at least in the laboratory), that it has been collected by the customer.
9.3 Qualitative analyses

In the case of qualitative analyses only, the results must be expressed as follows:

- positive (the analyte has been identified in accordance with the laboratory’s own analytical procedures);
- negative (the analyte has not been identified in accordance with the laboratory’s own analytical procedures).

9.4 Quantitative analyses

In the case of quantitative analyses, the result must be expressed using the appropriate unit of measurement (mg %, mg/mL, mg/gr, mcg/mg, mcg/mL etc). The result should always be expressed in numerical terms. However, whenever the positive identification concerns the presence of an analyte at a level of concentration that are lower than the lowest calibration point (but higher than the cut-off value foreseen for the confirmatory method) foreseen by the standard operational procedures, the word “trace” may be used as a semi-quantitative response.

9.5 Conservation of the analytical report

A copy of the final analytical report and of the supporting documents must be kept for at least 3 years, or for longer depending on the health regulations in force.