SOFT / AAFS

FORENSIC TOXICOLOGY LABORATORY GUIDELINES

2002

1. INTRODUCTION

The *Forensic Toxicology Laboratory Guidelines* were originally published in 1991 as two main documents (*Guidelines* plus *Appendix*), plus the self-evaluation checklist. The primary document, the *Guidelines*, was initially drafted in response to the growth and regulation of forensic urine drug testing. It was an attempt to take the important issues that were addressed for *Federal Workplace Drug Testing Programs* and draft them into terms which could be more realistically applied to the areas of *Post-Mortem Forensic Toxicology* and *Human-Performance Forensic Toxicology*. However, the Guidelines Committee agreed that there were many additional issues which were important to cover, but which might better belong in a supplementary document - the Appendix to the Guidelines. Since 1991, the profession has matured in many ways. In 1996 the American Board of Forensic Toxicology launched a Forensic Toxicology Accreditation program based primarily on the SOFT/AAFS Guidelines and Appendix. In 1997 New York State passed legislation requiring the accreditation of all forensic laboratories in the public sector, and others may follow. The Guidelines Committee concluded that it was time to redraft the original *Guidelines* and *Appendix* into a single cohesive document which would be easier to reference and to update in the future. That was done, and the final document approved and adopted. Subsequent changes to the format and content were made and approved in 1998, 2000 and 2002.

Introduction from 1991 Guidelines

In response to the Guidelines for Federal Workplace Drug Testing Programs issued by the U. S. Department of Health and Human Services in 1987, the Society of Forensic Toxicologists and the Toxicology Section of the American Academy of Forensic Sciences appointed a joint committee of members to recommend a supplementary set of guidelines for the practice of forensic toxicology. The federal guidelines, especially with respect to laboratory personnel and operating procedures, may not always be appropriate for other types of forensic toxicology, and the guidelines set forth below represent recommendations of the Society/Academy committee in response to that issue. These suggestions do not necessarily reflect opinions about the minimum requirement for any laboratory, and have no regulatory purpose; rather, they are intended to assist laboratories engaged in the practice of forensic toxicology in achieving future goals.

2. SCOPE

The original committee concluded that specific guidelines for the practice of forensic toxicology would be appropriate for two defined areas: **Post-Mortem Forensic Toxicology** and **Human Performance Forensic Toxicology**.

The committee concluded that it was *not* appropriate to include **Forensic Urine Drug Testing**, because that area of practice has been covered by the Department of Health and Human Services Guidelines and by the College of American Pathologists Accreditation program.

The specific aims of the committee, with respect to postmortem and human-performance

forensic toxicology, were to provide detailed guidelines for laboratory practices and to prepare a checklist for self-evaluation that may also serve as an important component of a program designed to prepare a laboratory for accreditation. The self-evaluation checklist has since been dropped, since it was adopted and expanded by the American Board of Forensic Toxicology as part of their laboratory accreditation program in 1996.

3. DEFINITIONS

Post-Mortem Forensic Toxicology - determines the absence or presence of drugs and their metabolites, chemicals such as ethanol and other volatile substances, carbon monoxide and other gases, metals, and other toxic chemicals in human fluids and tissues, and evaluates their role as a determinant or contributory factor in the cause and manner of death;

Human-Performance Forensic Toxicology - determines the absence or presence of ethanol and other drugs and chemicals in blood, breath or other appropriate specimen(s), and evaluates their role in modifying human performance or behaviour. (The analysis of ethanol in breath, although important, was not considered by the committee because such tests are not conducted in a laboratory setting); and

Forensic Urine Drug Testing - determines the absence or presence of drugs and their metabolites in urine to demonstrate prior use or abuse.

Standard - a reference material possessing one or more properties that are sufficiently well established that it can be used to prepare calibrators.

Calibrator - a solution , either prepared from the reference material or purchased, used to calibrate the assay. Where possible, calibrators should be prepared in a matrix similar to that of the specimens.

Control - a solution either prepared from the reference material (separately from the calibrators; that is, weighed or measured separately), purchased, or obtained from a pool of previously analyzed samples. Controls from any of these sources are used to determine the validity of the calibration; that is, the stability of a quantitative determination over time. Where possible, controls should be matrix-matched to specimens and calibrators, as indicated above.

Reference Material (RM) - a material or substance one or more properties of which are established sufficiently well to be used for calibration of an apparatus, assessing a measurement or assigning values to material. (AOAC Official Methods of Analysis (1984)).
Certified Reference Material (CRM) - a reference material, one or more of whose properties are certified by a valid procedure, or accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (AOAC Official Methods of Analysis (1984)).

4. PERSONNEL

4.1 Laboratory Director

4.1.1 The forensic toxicology laboratory should be directed by a person who is qualified by reason of appropriate education and experience to assume the required professional, organizational, educational, managerial and administrative responsibilities.

4.1.2 That education and experience should be comparable to those of persons certified as Diplomates by the American Board of Forensic Toxicology.

4.1.3 Alternative acceptable qualifications include a doctoral degree in one of the natural sciences and at least three years of full-time laboratory experience in forensic toxicology; or a Master's degree in one of the natural sciences and at least five years of full-time laboratory experience in forensic toxicology; or a Bachelor's degree in one of the natural sciences and at least seven years of full-time laboratory experience in forensic toxicology.

4.1.4 The director should also have documented training and/or experience in the forensic applications of analytical toxicology (such as court testimony, research, participation in continuing education programs, and/or peer review of appropriate manuscripts in the field), including a knowledge of evidentiary procedures that apply when toxicological specimens are acquired, processed, and stored and when toxicological data are submitted as part of a legal proceeding.

4.1.5 The laboratory director should be responsible for ensuring that the laboratory personnel are adequately trained and experienced to conduct the work of the laboratory

4.1.6 The laboratory director should be responsible for maintaining the competency of laboratory personnel by monitoring their work performance and verifying their skills. This training and experience should be documented.

4.1.7 The laboratory director should be responsible for the development of a complete, up-to-date procedures manual that is available to and followed by all personnel performing tests.

4.1.8 The laboratory director should establish a procedure for validating new analytical methodologies, and for maintaining a quality assurance program to ensure the proper performance and reporting of all test results.

4.1.9 Since forensic toxicology laboratories handle controlled substances and generate results essential to the criminal justice system, the director, to the extent practical or permitted by law, should exert reasonable efforts to ensure that all personnel meet high ethical and moral standards.

4.2 Other Laboratory Staff

The range and type of duties of other laboratory personnel will vary according to the size and the scope of the laboratory. It is recommended that each laboratory should have the following.

4.2.1 A person with the title of deputy director, assistant laboratory director, assistant chief toxicologist, or supervisory toxicologist, who has sufficient training and experience to be familiar with all administrative and testing procedures. He or she may supervise the work of all analysts, and should be capable of performing full scientific review of all test data, and of acting for the laboratory director in the director's absence. It is recommended that such individuals should have a minimum of a Bachelors degree in a natural science and 3 years of training in analytical toxicology, at least 1 year of which is in forensic toxicology.

4.2.2 One or more technicians who are capable of performing a variety of test procedures for alcohol, drugs, and other chemicals. A technician may supervise and review the work of less experienced technicians, and may supervise a section in a larger laboratory. It is recommended that such individuals should have a minimum of a Bachelor's degree in a natural science, at least 1 year of experience in analytical toxicology and 6 months experience in the present employment.

4.2.3 One or more analysts who are capable of performing tests for one or several analytes, and who are certified in each procedure by the laboratory director. These analysts may be limited in function to perform specified tasks - for example, an analyst who performs only immunoassays.

5. STANDARD OPERATING PROCEDURES

5.1 The laboratory should have a standard operating procedure manual (SOP) that is complete, up-to-date, and available to all personnel who are performing tests.

5.2 The SOP manual should include detailed descriptions of procedures for sample receiving, accessioning, chain-of-custody, analysis, quality assurance and quality control, review of data, and reporting.

5.3 The SOP manual should include administrative procedures as well as analytical methods and be reviewed, signed, and dated whenever it is first placed into use or changed.

5.4 The SOP manual should include, for each analytical procedure if appropriate, the following: a) theory and principle of the method, b) instructions for preparation of reagents, c) details of the analytical procedure, d) instructions for preparation of calibrators and controls, e) information about any special requirements for handling reagents or for ensuring safety, f) validation parameters (e.g. LOQ, linearity), g) criteria for the acceptance or rejection of qualitative or quantitative results and h) references.

5.5 When the required documentation is not available for infrequently performed assays, it should be added as each is performed for the first time.

5.6 The laboratory should maintain out-dated copies of the SOP manual and provide a means for their retrieval from archival storage.

6. SAMPLES AND RECEIVING

6.1 Specimen Collection and Labelling

The proper selection, collection, and submission of specimens for toxicological analyses is of paramount importance if analytical results are to be accurate and their subsequent interpretation is to be scientifically sound and therefore useful in the adjudication of forensic cases. These guidelines can apply equally to investigations by Medical Examiners or Coroners (postmortem forensic toxicology) and to investigation by law-enforcement agencies of drivers suspected of being under the influence of alcohol or other drugs.

6.1.2 The director should develop and provide detailed guidelines and instructions to all agencies or parties the laboratory serves.

6.1.3 Instructions should state the types and minimum amounts of specimens needed to accomplish the requisite analyses and subsequent interpretations.

6.1.4 Whenever possible, the amount of specimen collected should be sufficient to ensure that enough remains for subsequent re-analysis if required.

6.1.5 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.

6.1.6 Instructions for labelling individual specimen containers, and acceptable conditions for packing and transportation, should also be provided.

6.1.7 Submitting agencies should be instructed on how to label clearly all samples from living subjects or decedents who may carry a highly infectious disease such as tuberculosis, hepatitis or Human Immunodeficiency Virus.

6.1.8 Each specimen should be identified as to type. For blood, the anatomical site of collection should be stated. When antemortem and/or perimortem specimens are available from a decedent, each specimen should be labelled with the time and date of collection.

6.1.9 The name of the subject from whom the specimens were collected should appear on each label together with other appropriate identification; for example, the Medical Examiner's Case Number and/or the subject's Social Security Number.

6.1.10 Where provided, the time and date registered for each specimen should be initialled or signed by a responsible person who performed or witnessed the collection and who assumes

responsibility for the chain of custody.

6.2 Specimen Handling

6.2.1 A chain-of-custody form should be designed that will accompany specimens from the place of collection to the laboratory. This document may be incorporated in the laboratory-request form.

6.2.2 Handling and transportation of a specimen from one individual or place to another should always be properly documented.

6.2.3 The chain-of-custody section should be properly completed by responsible personnel at the time the specimens are collected.

6.2.4 Every effort should be made to minimize the number of persons handling a specimen.

6.2.5 Individual specimens should be transported and stored in such a manner as to minimize the possibility of degradation, contamination, tampering and/or damage in shipment.

6.2.6 The condition of the external package should be documented upon receipt at the laboratory, either on the requisition form that accompanies the specimen(s), in the log book, on the external chain-of custody form, or on other documents that constitute normal laboratory records.

6.2.7 Acceptable means of transporting specimens to the laboratory may include hand-delivery, national postal service, or a private or government courier service.

6.3 Specimen Receipt

6.3.1 The means of delivery of specimens should be recorded by the receiving laboratory.

6.3.2 Shipping containers should be opened only in a secure area and only by an individual designated to record receipt of specimens. A "secure area" may be defined as an area to which unauthorized individuals do not have access without escort by authorized personnel.

6.3.3 A hard copy of the specimen-receipt record should be permanently maintained; this record may be computer-generated, typed, or hand-written.

6.3.4 Specimens should be logged-in immediately upon receipt.

6.3.5 The integrity of the individual specimen container should be checked as should the condition of each specimen. Discrepancies should be recorded.

6.4 Recommended Amounts of Specimens

6.4.1 Post-Mortem Forensic Toxicology Specimens: In death investigations, the types and minimum amounts of tissue specimens and fluids needed for toxicological evaluation of the role of drugs and other toxic chemicals are frequently dictated by the analyte or analytes that must be identified and quantitated. For example, in the evaluation of carbon monoxide poisoning, 10 mL of whole blood would be sufficient with adequate specimen volume remaining for re-analysis if required. On the other hand, to evaluate the role of amitriptyline in a death, 25 gm of liver, 10 mL of heart blood, 5 mL of peripheral blood and the entire gastric contents should be made available.

Many deaths involve ingestion of multiple drugs, necessitating larger amounts of tissue and fluids to be collected at autopsy for toxicological examination. The following is a suggested list of specimens and amounts to be collected at autopsy in such cases:

Brain	100 gm
Liver	100 gm
Kidney	50 gm
Heart Blood	25 mL
Peripheral Blood	10 mL
Vitreous Humor	All Available
Bile	All Available
Urine	All Available
Gastric Contents	All Available

Unique poisons may dictate the need for other specimens, e.g. lung and intestine. Such cases should be addressed on an individual basis. However, the amount of specimen routinely collected should be sufficient to allow re-analysis for one or more analytes at a later time, should the need arise.

6.4.2 Human Performance Forensic Toxicology Specimens: As defined earlier, this activity encompasses the identification and quantitation of ethanol and other drugs and chemicals in blood, breath or other appropriate specimens for evaluation of their role in modifying human performance and behaviour. The analysis of breath ethanol was not considered by this Committee.

Although in many instances the analytes are clearly specified in advance in human performance forensic toxicology testing, the spectrum of drugs and chemicals may potentially approach those encountered in postmortem toxicology. Because of this and remembering the difficulties involved in obtaining samples from living persons, it is recommended that a minimum of 15 mL of blood be collected for toxicological analysis.

Urine may also be submitted for testing; a minimum volume of 30 mL is recommended. It must be emphasized that neither qualitative nor quantitative analysis of urine permits an evaluation of the effect of the drug or chemical on human behaviour. If other specimens are submitted and analyzed, any conclusions regarding drug use or effects on human behavior should be based only on appropriate validated scientific studies.

7. SECURITY AND CHAIN-OF-CUSTODY

7.1 The Laboratory

7.1.1 Access to the forensic toxicology laboratory should be limited. The laboratory director should authorize and document the personnel able to enter designated areas.

7.1.2 Unauthorized personnel should be escorted and may be required to sign a log-book upon entry and departure from the laboratory, recording the time, date and purpose of the visit.

7.1.3 The physical layout of the laboratory must be such that unauthorized personnel cannot enter without detection.

7.2 Specimens

7.2.1 Receipt of specimens should be indicated by signature, and date and time of individuals receiving the specimens.

7.2.2 Specimens received should be labeled with the name of the decedent or suspect, case number, specimen type (e.g. blood) or unique identifier, date specimen taken and identification of the individual taking the sample.

7.2.3 Specimens must be stored in a secure manner.

7.2.4 For the maintenance of specimen security it is recommended that, where possible, the laboratory have a separate accessioning area. In this area, specimens are received, assigned accession numbers, aliquots removed and/or stored in refrigerator/freezers.

7.2.5 Any transfer of specimens, or portions thereof that are removed for analysis, must be documented as part of the permanent laboratory record.

7.2.6 It is recommended that chain of custody documentation reflects not only the receipt of the specimen from an outside source, but also transfers of the specimen or an aliquot thereof, within the laboratory. If multiple specimens are involved, a batch form may be used.

7.2.7 An aliquot or a batch of aliquots chain of custody may be used for indicating the transfer of portions of specimens for testing. This form should indicate the date, the test for which the aliquot was taken, the laboratory accession numbers, the identity of the individual obtaining the aliquots and the identity of the individual to whom the aliquots were given, if applicable.

7.2.8 Specimens may be transferred to a secure long-term refrigerator/freezer after analysis. Transfers between storage areas and/or subsequent disposal should be documented. The laboratory should develop a standing operating procedure for retention and disposal of

specimens. This procedure should reflect local, state, or federal regulations.

7.2.9 The laboratory should maintain a written policy and instructions pertaining to retention, release and disposal of specimens.

8. ANALYTICAL PROCEDURES

8.1 Confirmatory Tests

8.1.1 As a general matter of scientific and forensic principle, the detection or initial identification of drugs and other toxins should be confirmed whenever possible by a second technique based on a different chemical principle.

8.1.2 Where possible, the confirmatory (second) test should be more specific and sensitive than the first test for the target analyte. The use of mass spectrometry is recommended as the confirmatory technique, where practical.

8.1.3 In some circumstances, confirmation using the same GC system as the first might be acceptable if chemical derivatization (e.g. silylation or acylation) is used to change the retention times. However, confirmation using a second GC system with a similar though not identical column, is not usually acceptable since the retention indices of many analytes may not differ substantially from one system to the other (e.g. DB-1 and DB-17).

8.1.4 Use of a second immunoassay system (e.g. RIA) to confirm another immunoassay (e.g. FPIA) is not regarded as acceptable, even though the assays differ somewhat in principle. The rationale for this is that the analytes which cross-react with one assay are also likely to cross-react in the second assay because the antibodies may be raised to the same drug or closely related substance.

8.1.5 A second immunoassay with different cross-reactivity may sometimes be used to augment the initial screen (for example a broadly cross-reacting opiate immunoassay, followed by a second immunoassay with more specific cross-reactivity to unconjugated morphine). These results would normally still require confirmation with a more specific method (e.g. GC/MS).

8.1.6 It is a good practise to confirm the identity of an analyte in a different extract of the same specimen from that used for the first test, or in a second specimen. However, confirmation of a drug or toxin in the same original extract of a single specimen would not normally be regarded as acceptable, since that would not rule out the possibility that the extract became contaminated during the extraction.

8.1.7 The quantitation of an analyte may serve as acceptable confirmation of its identity if it was initially detected by a significantly different method (e.g. GC/MS SIM quantitation of a drug detected by immunoassay).

8.1.8 Where mass spectrometry is used in selected ion monitoring mode for the identification of an analyte, whether as part of a quantitative procedure or not, the use of at least one qualifying ion for each analyte and internal standard, in addition to a primary ion for each, is strongly encouraged where possible. Commonly used acceptance criteria for ion ratios is ±20% relative to that of the corresponding control or calibrator. However, it is recognized that some ion ratios are concentration dependent and that comparison to a calibrator or control of similar concentration may be necessary, rather than an average for the entire calibration. Ion ratios for LC/MS assays may be more concentration and time dependent than for GC/MS and therefore acceptable ion ratio ranges of up to ±25% or 30% may be appropriate.

8.1.9 GC/MS chemical ionization and LC/MS mass spectra are often simpler than GC/MS EI spectra and therefore afford less options for the choice of qualifier ions. However, it is often possible to adjust the ionization energy (e.g. cone or fragmentor voltage in LC/MS) in order to produce additional or stronger secondary ions. Running the sample under conditions of both weak ionization (to maximize the quantitation ion signal) and stronger ionization (to promote fragmentation and facilitate confirmation of identity) is an option.

8.1.10 It is recommended that at least the presence of a drug or toxin be verified in more than one specimen, or if only one specimen is available by replicate analyses on different occasions and with adequate positive and negative controls in the same matrix. However, it is acknowledged that an analyte will not necessarily be present in all specimen types.

8.1.11 Use of a second confirmatory technique is encouraged for all analytes, including ethanol (e.g. GC, ADH, or colorimetric) and carbon monoxide (e.g. visible spectrophotometry, palladium chloride or GC).

8.1.12 It is recognized that in some circumstances a suitable second test procedure is not available and the probability that the first test is incorrect is almost zero. For example, the probability that a 75% carboxyhemoglobin in a well-documented suicide is incorrect, when obtained by a properly conducted spectrophotometric assay, is exceedingly low. However, the unexpected finding of a 30% carboxyhemoglobin from a motor vehicle accident victim by a similar determination in blood holds a lower degree of certainty.

8.2 **Method Calibration and Validation**

8.2.1 When conducting analyses, laboratories may group specimens into batches. Each batch should contain a sufficient number of calibrators and controls, the total number of which will depend on the size of the batch and the nature of the tests.

8.2.2 When analyses are being performed on unusual specimens (decomposed tissue, vitreous fluid, etc.), appropriate matrix-matched calibrators should, when possible, be prepared and tested concurrently with the specimens.

8.2.3 For immunoassays, a laboratory should, at a minimum, be able to demonstrate that the blank or negative calibrator plus two standard deviations does not overlap with the cut-off or the lowest positive calibrator. Alternatively, the laboratory may determine the limit of detection (LOD) by determining the mean value for the blank and adding three standard deviations to this value (LOD = Xm + 3SD). However, it should be noted that for other assays (e.g. GC, HPLC) the true LOD may be higher than indicated by this formula if significant adsorption or other losses occur. For example, in chromatographic assays, the LOQ might be the smallest blood concentration of a drug needed to give a peak height three times the noise level of the background signal from a blank blood sample. Alternatively, for infrequently performed assays where the analyte measured is always within the calibration range of the assay and well above the LOD, it may be sufficient to indicate that the detection limit is “less than” a certain value. Thus the true LOD may be derived experimentally, but should not be less than the blank plus three standard deviations. The limit of quantitation (LOQ) may be derived by adding ten standard deviations to the true value of the blank. However, it is preferable to determine the LOQ experimentally as the lowest concentration for which an acceptable coefficient of variation can be routinely achieved.

8.2.4 For chromatographic assays, the LOD and LOQ may be administratively defined in terms of the concentration of the lowest calibrator, and therefore may not need to be determined experimentally. However, if results are reported below the value of the lowest calibrator, LOD and LOQ should be determined.

8.2.5 The use of a suitable internal standard for all chromatographic assays (e.g. GC, HPLC, GC/MS) is recommended. The internal standard should have chemical and physical properties as similar to the analyte as possible. If the analyte is to be derivatized, an internal standard should be chosen which will form an analogous derivative. Stable isotope (e.g. deuterated) standards are recommended for GC/MS assays, although well chosen non-deuterated internal standards may occasionally give equivalent or better performance. The internal standard should be added to the sample at the earliest possible stage in the method, and in any event before buffering and extraction of the sample. Markers which are added after the initial extraction are regarded as "external standards" and are discouraged.

8.2.6 Linearity of the procedure should be established by typically using at least three calibrators. The concentration of the calibrators should be such that they bracket the anticipated concentration of the specimen(s). If the concentration of the specimen exceeds the concentration of the highest calibrator, the specimen should be diluted and re-extracted if accurate quantitation is required. Otherwise the specimen should be reported as having a concentration greater than the highest calibrator. If the concentration of the specimen should be less than that of the lowest calibrator, an additional calibrator should be set up which falls below the expected range of the analyte in the sample. Alternatively, the volume of the specimen may be doubled and re-extracted if it can be demonstrated that the assay is not matrix dependent. If an accurate quantitation is not necessary, then the specimen can be reported as containing the analyte at less than the lowest calibrator (as an alternative to the term "trace amount"). It is acknowledged that some assays are inherently non-linear and that the use of quadratic or other mathematical models may be necessary.

8.2.7 Criteria for acceptance of a chromatographic calibration should be stated in the method. For a multi-point calibration this factor is usually the correlation coefficient. For most applications, an acceptable correlation coefficient is 0.99. However, there may be circumstances where a correlation coefficient of 0.98 is minimally acceptable. In addition, it is good practice to evaluate the range of the calibration by calculating the value of each calibrator against the curve. Values of ±20% are generally acceptable for most applications, although ±10% are preferred for analytes such as ethanol. Single point calibrations are discouraged unless controls are used at or close to the upper and lower quantitative reporting limits.

8.2.8 For specimens having concentrations significantly higher than the highest calibrator, the laboratory should exercise precautions so that carry-over of analyte into the next specimen does not occur. Similarly, specimens with very low concentrations should be checked to ensure that carry-over from a previous very high positive has not occurred.

8.2.9 It is recognized that for a variety of reasons occasional analytical results will be "outliers"; that is, analytical values which deviate significantly and spuriously from the true value. "Outlier" results of control, blanks or calibrators should be obvious. However "outlier" results of case specimens may not be identified if only run singly, unless that result can be compared with one from a separate analytical determination. For this reason replicate extraction and quantitative analysis, at least in duplicate, is recommended. The laboratory should determine the acceptable criteria for replicate analysis. A maximum deviation of ±20% of the mean is recommended.

8.2.10 Retention time should be part of the acceptance criteria for chromatographic assays. For GC based assays, deviations of 1 - 2% from the calibrators or controls may be acceptable. Slightly larger deviations may be acceptable for HPLC based assays, particularly where the mobile phase is being programmed non-isocratically.

8.3 **Method of Standard Additions**

It is recognized that the matrix of some forensic specimens may be "unique" in some way (e.g. putrefied or embalmed) such that it is difficult or impossible to obtain a similar matrix for the preparation of reliable calibrators and controls. In these circumstances, the use of a "standard addition" procedure may be preferable to a conventionally calibrated assay. In the method of "standard addition" known amounts of analytes are added to specimen aliquots and quantitation performed by comparing the proportional response of the fortified aliquots with that of the unknown specimen. Use of an internal standard and a multiple point calibration is strongly recommended.

9. QUALITY ASSURANCE AND QUALITY CONTROL

9.1 **Quality Assurance**

9.1.1 Quality assurance encompasses all aspects of the analytical process, from specimen collection and reception through analysis, data review and reporting of results. It includes, but should not be limited to, quality control of each analysis and proficiency testing of the laboratory.

9.1.2 Quality assurance assumes a unique role in the forensic science disciplines because results are subject to challenge in the "adversarial" justice system. One purpose of a quality assurance program is to detect error, whether random or systematic, and to initiate appropriate remedial action.

9.1.3 Standards used should be appropriate for the test being performed, and documentation should be maintained describing their sources and dates of acquisition. Reference material should be stored so as to ensure its stability and integrity. If a standard is prepared in the laboratory, the source(s) of the chemical reagent(s), the method of preparation, and verification of the final product should be recorded and maintained on file.

9.1.4 Where practical, the identity and purity of reference materials should be verified by the laboratory.

9.1.5 Labelling should be uniform for all standards and reagents. Date of acquisition or preparation, and the initials of the preparer, should be included on the label. The expiration date should always appear on the label. An expiration date furnished by a vendor/manufacturer determines the useful lifetime of the standard/control unless it can be verified beyond that date.

9.1.6 Initially, a sufficient number of calibrators should be run to determine the characteristics of the calibration curve; a blank and at least three calibration points are recommended for the initial calibration process. The stability of the calibration curve should be tested under laboratory conditions by the addition of controls, both positive and negative.

9.1.7 Controls are not analyzed for calibration purposes. As a general rule an adequate set of controls should include, at a minimum, a specimen that does not contain the analyte (defined as a negative control) and a specimen containing the analyte at a concentration that realistically monitors the performance of the assay. Additional controls can be used to test the linearity of the calibration over the desired range.

9.1.8 The SOP manual should specify corrective action to be taken when control results are outside acceptable limits. Under optimal conditions a laboratory should have a quality control supervisor, but having a staff member dedicated to quality control may be impractical for small laboratories.

9.1.9 Forensic toxicology laboratories should participate in an external proficiency testing program which includes, at a minimum, a proficiency testing program for alcohol in blood or serum, and for drugs in at least one type of specimen; the program should realistically monitor the laboratory's quantitative capability.

9.1.10 The laboratory director should regularly review results of quality control and proficiency testing. Signing and dating the record constitutes appropriate evidence of review. It is important that bench personnel be informed of quality control and proficiency test results. Attention should be given to procedures for monitoring potential sources of error. Proficiency test materials should be retained until the summary report is received and any corrective action satisfactorily completed.

9.1.11 Appropriate and timely corrective action in the event of proficiency test errors is essential. False positive errors are the most serious and possible causes of the error must be thoroughly investigated, including contamination of glassware and carry-over. A false negative result can be defined as failure to detect a substance which the laboratory claims to be able to detect, or that should have been detected by the method. By this definition, a false negative indicates a failure that should be investigated expeditiously. A false negative can also occur because the routine methods of the laboratory will not detect the analyte at all, or at the spiked concentration. In this instance the laboratory director should decide whether the analytical procedures need revising, or whether the failure to detect that analyte at the spiked concentration is acceptable (e.g. the concentration is below that of toxicological interest). All corrective action should be documented.

9.1.12 Quantitative proficiency test errors should also be investigated. Usually, the target concentrations of analytes are expressed in terms of the mean value for all participants in the survey, plus or minus 1 SD or 2 SD. Occasionally, the weighed-in target may be disclosed. Where the magnitude of an error is large, the need for corrective action is obvious and the underlying cause may be easy to determine. For some analytes, especially those infrequently quantitated, 2 SD, a common measure of acceptability, may represent an unacceptably large percentage deviation from the mean. Therefore, a realistic percentage deviation should be used, such as ±20% or ±30%. Depending on the magnitude of the error, corrective action may be as simple as review of the assay results to ensure that the calibration was valid, that the assay was in control, and that any transcriptions were accurate. For more serious errors, corrective action may require repeating the analysis, re-validation of the assay, or even redevelopment of the test. All corrective action should be documented.

9.1.13 Routine maintenance of equipment is an important part of any quality assurance program. It is a good practice to document all routine and non-routine maintenance, including tasks such as changing septa and liners on GCs. Documentation may be in a logbook, which can be kept by larger equipment, or check-sheets filed in a ring binder. Multiple items of similar equipment (e.g. pipettors) should be labelled in order to readily differentiate them.

9.2 **Quality Control**

9.2.1 Control Materials: In the true sense, a control is a test sample, identical to the unknown, but containing the analyte at a known concentration. With each batch of specimens, whether a single specimen or multiple ones, controls would be carried through the procedure in parallel with the unknowns. It is suggested that each batch of specimens include at least 10% controls. The controls must include one positive and one negative control. For qualitative assays positive

and negative controls, acceptable results may simply be “positive” or “negative”, respectively. For quantitative assays, negative controls should give results that indicate the analyte is absent, or below the LOD for the assay. An acceptable positive control result of ±20% is recommended for most drugs, except for controls that are at or close to the LOQ of the assay, when ±25-30% may be more realistic. The control must give a result within a predetermined deviation from its mean value, or the test is deemed "out of control" and therefore, the result generated from the unknown specimen is unacceptable.

9.2.2 It is a common and accepted practice in clinical laboratory work to obtain or prepare material and then establish the target range by replicate analysis of the control in parallel with existing QC material. For example, control material may be prepared by pooling specimens from multiple cases. While that approach is still accepted in forensic toxicology, it is scientifically less desirable than preparing or purchasing control material with a specific weighed-in target concentration, which will allow independent verification of calibration accuracy. If control target ranges are experimentally determined, it is important for that range to be verified against control material, prepared commercially or independently in-house, prior to it being put into routine use.

9.2.3 For some forensic toxicology procedures, providing a true control is no more difficult than any other test. For others, however, in which the matrix may be unique (e.g. decomposed tissues, bone, hair or nails) , providing a control is not only difficult, but can never approach the ideal of being identical to the unknown specimen. Controls should be prepared from standard material from a different source than that used in calibration of the assay. Where this is not practical, the control should at least be prepared using a different weighing or dilution than that used to prepare the calibrators. Control material prepared from the same solution used to prepare the calibrators is unacceptable, since any errors made in preparation of the standard solution will not be detected.

9.2.4 Open Controls: Open controls are those whose identity and expected result are known to the analyst. They can be purchased from commercial vendors, prepared in the laboratory, distributed by professional organizations or saved and pooled from former cases. Regardless of the source, the concentration of the analyte in the control must be validated.

For tissue specimens or other unusual matrices, more innovative approaches may be necessary. Fortifying drug-free matrices, such as tissue homogenates, out-dated blood bank blood, plasma to simulate the unknown specimen is acceptable. A "blank" or negative control may, of course, be the unfortified matrix.

9.2.5 Results from quantitative quality control material should be recorded in a manner that readily permits the detection of trends such as the deterioration of reagents, calibrators or controls. For frequently run controls, results may be plotted in a graphical manner such as a Levy-Jennings plot. For less frequently run material, tabulation is acceptable. Determination of the coefficient of variation for the controls may give useful information about the precision of the assay, and may indicate which assays need further development.

9.2.6 Blind Controls: As the name implies, these are identical to open controls except their identity is unknown to the analyst. It is generally recognized that this is the ideal way to maintain quality control. A blind control should test the entire laboratory process including receiving, accessioning, analysis and reporting. This can be accomplished by setting up a "dummy account" or by co-operation with the submitting agency. Such blind controls are sometimes called "double blinds". A more practical approach is to have the accessioning section insert blind controls into each batch of specimens. However, either of these processes can be difficult to accomplish in a small laboratory; they are both costly and time consuming.

9.2.7 Sources of Information: There are many good references relating to the general topic of quality control as well as to specific application relating to analytical procedures. These include: *Tietz Textbook of Clinical Chemistry*, 3rd edition, by Carl A. Burtis, Edward R. Ashwood, Norbert W. Tietz, Adrianne Williams (Editors), W.B. Saunders Co., Philadelphia, 1998; *Cost-Effective Quality Control: Managing the Quality and Productivity of Analytical Processes*, by James O. Westgard and Patricia L. Barry, AACC Press, Washington DC, 1986; *Quality Assurance in Postmortem Toxicology* by Wilmo Andollo, Chapter 12.5 in *Handbook on Drug Abuse*, Steven Karch senior editor, W. Lee Hearn section editor, CRC, Boca Raton, FL, 1998, pp 953-969.

9.3 **Reference Materials**

9.3.1 The National Institute of Standards and Technology (NIST), formerly the National Bureau of Standards (NBS) , refers to these as Standard Reference Material (SRM). For example, a specific RM may have a melting point of such sharpness and reproducibility that it can be offered as an RM for the calibration of a thermometer in a melting point apparatus. However, it may not be appropriate for preparing a calibration curve. A CRM, or SRM, suitable for the preparation of a standard to which calibration material can be compared, must be certified by a method generally recognized by the scientific community as one which validates the CRM for this purpose. The nature of the procedure depends, of course, on the properties of the analyte.

9.3.2 Several different organizations supply CRMs of a variety of types. A complete listing of organizations supplying CRMs is available from the International organization for Standardization, American National Standards Institute, 1430 Broadway, New York, NY 10018. Catalogs or literature describing CRMs are available from individual organizations, such as the NIST.

9.3.3 It is important to remember that most RMs are not 100% pure. The label or package insert should indicate the purity or the nature of the contaminants or the degree of water of hydration. Further instructions may provide guidance as to how the RM is to be used. For example, perhaps it must be protected from light, or stored at a low temperature or protected from moisture. These instructions must be carefully followed in order to use the RM according to its specifications.

9.3.4 Drugs, perhaps more than many other chemicals, may have limited shelf-lives. Degradation due to photo-reactions, oxidation in the air or by other means, requires that periodic

assessment of these changes must be monitored. Methods for detecting such changes are varied but even RMs may not retain their original purity. RMs supplied in solution may have more limited stability than those supplied as pure, dry, solids.

9.3.5 The importance of acquiring pure chemicals used as standards and periodically monitoring their purity, requires the development and implementation of procedures which are part of the standard operating procedure of the laboratory. The steps which can be used are summarized as follows:

1) maintain instruments and all measuring devices at optimal performance with regular calibration checks.
2) acquire chemicals to be used as standards from reliable sources who validate the stated purity, preferably by a certifiable trace to a CRM or SRM, or
3) acquire chemicals as RM, carefully following any instructions accompanying the RM for maintaining anhydrous conditions or to avoid deterioration, or
4) acquire chemicals from other sources but always assess the purity of the material by appropriate measurement of physical constants and/or instrumental methods.
5) regardless of the source of the chemical for preparation of the standard, devise a means by which the standard can be monitored periodically in order to detect any deviation from its original purity.
6) before using a newly prepared standard, compare its properties with a previously validated standard or with a CRM or SRM.

9.4 **Metabolites**

9.4.1 Many testing procedures, particularly immunoassay tests, are targeted to detect drug metabolites. As might be expected, these are more difficult to obtain in pure form, free of interferences and certified as to their authenticity. A number of commercial sources offer drugs and some metabolites, together with deuterated forms useful as internal standards in GC-MS. Frequently the commercial sources will supply a statement of purity with the material. This is not the same as a CRM or SRM, but after verification of purity, may be quite acceptable.

9.4.2 Metabolites of pharmaceutical drugs can, at times, be obtained from the company which manufactures them. This often requires a personal contact with an appropriate official of the company, completion of necessary paperwork and some time delay. The *Physicians' Desk Reference* in its "Manufacturers' Index" lists names and telephone numbers of contact officials.

9.4.3 When the identity of the metabolite has been described in a reputable scientific journal, but no source is evident, a search of catalogs from suppliers of organic chemicals may be fruitful. If this is not successful, then it may be necessary to synthesize the metabolite. In this case its identity should be confirmed by standard, acceptable methods. In all of these alternatives, purity must be assessed.

10. REVIEW OF DATA

10.1 Before results are reported, each batch of analytical data should 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 (e.g., shape and signal-to-noise ratio of chromatographic peak) and calculations
* quality control data.

10.2 Where possible, the results should be reviewed in the context of the case history, autopsy findings and any relevant clinical data. The review should be documented within the analytical record.

11. REPORTING OF RESULTS

11.1 General Recommendations

11.1.1 Many, if not most, forensic toxicology laboratories are an integral part of state or local government supported, medico-legal investigative agencies, or are associated with them. Each laboratory must follow the mandates of the particular agency and/or governmental sub-division when reporting results. Thus, while it is neither possible nor desirable to suggest a uniform format for reports, they should include all information necessary to identify the case and its source, and should bear test results and the signature of the individual responsible for its contents.

11.1.2 The following recommendations are made:

1) name and/or identification number
2) laboratory identification number
3) name of submitting agency or individual
4) submitting agency's identification number
5) date submitted
6) date of report
7) specimens tested
8) test results
9) signature of approving individual

11.1.3 Although most forensic toxicology reports are confidential and often sensitive in content, some jurisdictions may treat the report as an official public document. If the results are confidential, every precaution should be exercised to ensure that a properly authorized person receives the information when it is transmitted by telephone, computer, FAX, or any other method different from conventional delivery of a written report. Each laboratory should formulate its own policy for retention or release of information and for response to requests for

its documentation.

11.2 Terminology in Reports

11.2.1 "Positive" indicates that a particular substance has been identified in accordance with the laboratory protocols. "Negative", "Not Detected", or "None Detected" has been generally used to indicate the absence of an analyte or analytes. "None detected" is preferable. This indicates that particular substances were absent within the limitations of the test(s) performed.

11.2.2 Tests may be described in a number of ways, individual chemical entities, groups or classes of chemicals or combinations of drugs or chemicals. A description of the entity should appear in the laboratory's standing operating procedure manual. This description should include the limitations of the test, such as the drugs included, the limits of quantitation, cut-off for the drugs included, cut-off concentrations (if applicable) or other terms to describe the lowest concentration reliably measured and reported in the specimen.

11.2.3 There may be both qualitative and quantitative results on a report. Qualitative results should be indicated by naming the test followed by positive or none detected. The term “trace” or a non specific numerical designation (e.g. positive but less than 0.5 mg/L) may be used if a substance was detected in a sample, but the concentration was less than the lowest point on a calibration curve or a designated cut-off.

11.2.4 Quantitative results should be identified using appropriate nomenclature (see #4). No quantitative value should be reported from a non specific immunological or other initial testing procedure, unless the procedure has been appropriately validated through parallel studies with a reference quantitative method.

11.2.5 Preferred units include mg/L, mcg/L, mg/Kg for fluids and tissues. Other units have been frequently used such as mg/dL, mg%, ng/mL, mcg/mL, mg/100 gm etc. Such terms may be appropriate, but laboratories should strive for the use of common terms on a national basis. Ethanol should be reported as percent (grams per 100 mLs.) Other commonly accepted units for certain analytes should continue to be used, such as mg/dL for glucose and meq/L for metals.

11.3 Preliminary Report

Although generally discouraged, issuing a preliminary report may be required before toxicology testing is complete. If that is done, only confirmed results should be released, or a clear statement included that the results are unconfirmed and subject to verification. The report should also include a statement that testing is incomplete, and where appropriate, that subsequent results may affect the final report and its interpretation.

11.4 Revised, Supplemental or Addendum Report

After the final report has been issued, it may be necessary to perform additional tests, in which case an addendum or revised report should be issued. These tests can be added to the existing

report, a revised report may be issued and so identified, or an addendum may be created to provide the results of the additional tests. Such a report should contain the same identifying information as the original report.

11.5 **Oral Reports**

Occasionally, it may be necessary to provide information on a report to a police or other external agency. In such situation, the results may be transmitted by telephone subsequent to ensuring that the individual is appropriately identified, that tests are recorded and the results reviewed.

11.6 **Corrected Reports**

After the final report has been issued it may become necessary to correct an error, typographical or otherwise, in the original or supplemental reports. In this instance the report should be clearly labeled as corrected and contain the same identifying information as the original report(s).

11.7 **Release of Reports**

There should be a procedure in the SOP manual for sending a report to the submitting agency.

11.8 **Referred Tests**

When samples are forwarded to other laboratories for analysis, there should be a record on the final report indicating this fact. Results of referred tests may be incorporated into the laboratory's final report.

11.9 **Retention of Records**

Records should be retained as long as practical, but for at least 5 years. Records should include a copy of the report, request and custody forms, work sheets, laboratory data, quality control and proficiency testing records.

There may be state or local regulation governing the time period over which records must be retained. Laboratory directors are advised to check with the appropriate agencies in their jurisdictions for information.

11.10 **Litigation Packs**

Laboratories are periodically asked to provide a copy of data and documentation related to a particular toxicology report or individual result. In North America, that is often called a litigation package, and is generally requested by a lawyer for review in a civil or criminal case. It should contain sufficient material to allow independent review by a qualified toxicologist. The

requesting lawyer or court order may dictate what is included in the package. However, it will typically include copies of the request for analysis, and chain of custody documents which track the sample from the time of receipt in the laboratory, through analysis and subsequent disposition of the sample(s). If requested, it may include all analytical data which supports identification, and if applicable, quantitation of the analyte(s). Where appropriate, it should include not just the raw data and reports, but worksheets, sequence tables, quality control data including target ranges. The material in the litigation pack should be complete and properly organized to facilitate review. For larger packs of material, it is helpful to provide a table of contents, and as necessary supplementary explanation.

12. SAFETY

The laboratory should have a safety manual that addresses at a minimum the following issues:

* specimen handling, including the handling of infectious material and the disposal of biological specimens
* handling and disposal of solvents, reagents, and other chemicals in the laboratory
* handling and disposal of any radioactive materials used in the laboratory
* handling and disposal of laboratory glassware
* responses to personal injuries and spillage of biological specimens, chemicals, solvents, reagents, or radioactive materials
* regulation governing dress (e.g. laboratory coats and safety glasses), eating, drinking, or smoking in the laboratory.

Each laboratory must be aware of State and/or Federal Regulations that may exceed minimum standard established on the basis of the above considerations.

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The Guidelines may only be modified by the Laboratory Guidelines Committee of the Society of Forensic Toxicologists and the Toxicology Section of the American Academy of Forensic Sciences as approved by the voting membership of both groups.