American Board of
ABFT
Forensic Toxicology ™

FORENSIC TOXICOLOGY LABORATORY ACCREDITATION MANUAL

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Section A: MANAGEMENT AND ADMINISTRATION

A-1 **E** **Does the laboratory have a written statement of its mission or objectives?**

The laboratory should have a brief statement, typically at the front of the SOP, outlining their primary activities. For example, this may be to provide a medical examiner or coroner system with comprehensive toxicology services that will assist in determining the cause and manner of death. Some laboratories may also provide support services for law enforcement agencies by providing analyses for alcohol or other drugs in biological fluids seized from motor vehicle drivers, other transportation operators, or from victims or those accused of crimes.

A-2 **I** **Is the laboratory provided with the financial resources to fulfill its mission**?

This question highlights an important consideration, but one which may be difficult to assess. One consideration is whether the laboratory clearly has insufficient staff or equipment to effectively fulfill its primary mission to generally acceptable minimum standards. However, it should also be considered whether a serious backlog of work might be due to staff shortages or equipment breakdowns that are temporary in nature. However, is not reasonable to expect a laboratory to be funded excessively for an inefficient workflow or for equipment that may be required for only a few specialized analyses.

A-3 **I** **Do laboratory staff have access to the necessary forensic, medical and other scientific literature?**

Toxicology staff should have reasonable access to up-to-date forensic texts. Commonly used texts should be available within the laboratory. This should include a compendium of analytical data for common drugs, basic pharmacology and toxicology texts and a compendium of prescription drug monographs. Examples might include *Disposition of Toxic Drugs and Chemicals in Man* (Baselt), *Clarke's Isolation and Identification of Drugs, The Pharmacological Basis of Therapeutics* (Goodman & Gilman), *Clinical Toxicology of Commercial Products,* and the *Physicians Desk Reference* (PDR). Staff should have reasonable access to the common forensic and analytical toxicology journals through subscription, university affiliation or electronic means.

A-4 **D** **Does the laboratory director hold regular meetings or communicate with staff by other means to keep them informed of changes to methods or procedures?**

It is important that there is effective and regular communication between the laboratory director and all other laboratory staff. In some laboratories this may be accomplished by holding regular (e.g. weekly, monthly) meetings. However, this may be difficult to arrange in busy laboratories, especially where more than one shift is in operation, or where staff are frequently away at court. Alternate acceptable means are via personal or posted memorandum. Regular (e.g. monthly) face-to-face meetings should occur between the laboratory director and staff responsible for QA/QC.

A-5 **I** **Is there an organization chart of the laboratory that includes reporting responsibility with regard to QA/QC issues?**

It is important for the laboratory to have an organizational chart or other means to clearly define the reporting structure of the laboratory, including whom QA/QC staff is responsible to.

A-6 **I** **Does the laboratory have a written policy that addresses the confidentiality of client information and results?**

A written policy should exist which addresses the confidentiality of laboratory data. This should include both the storage and release of information to third parties. While releasing results by telephone is not necessarily forbidden, reasonable precautions should be taken to prevent release by and to unauthorized persons. The exact precautions taken will depend on the jurisdiction and, for example, how well staff knows the police or other requesting agencies. Guidelines should also be given as to the extent of interpretation which may be given with the results and who is authorized to give that interpretation.

A-7 **D** **Is there a procedure to address the resolution of client complaints against the laboratory?**

From time to time, complaints may be received against a laboratory, covering everything from slow turnaround times, questioned accuracy or inability to conduct certain tests. A policy should be in place that requires an appropriate, documented response to all complaints received in writing. When necessary, corrective action should be taken and documented.

Section B: PERSONNEL

B-1 **E** **Does the laboratory have a Director with the required experience and qualifications?**

The forensic toxicology laboratory shall be directed by a person who has the appropriate education and experience to assume the required professional, organizational, educational and administrative responsibilities. The Director's experience and qualifications should to be comparable to those certified as Diplomates by the American Board of Forensic Toxicology.

Examples of alternative acceptable qualifications:

- Doctoral degree in a chemical or biological discipline and at least three years of full-time laboratory experience in forensic toxicology

- Master's degree in a chemical or biological discipline and at least five years of full-time laboratory experience in forensic toxicology

- Bachelor's degree in a chemical or biological discipline and at least seven years of full-time laboratory experience in forensic toxicology.

B-2 **I** **Is documentation of the Director's education and experience maintained?**

Examples of acceptable supporting documentation of Director's experience and qualifications include: an up-to-date curriculum vitae; up-to-date list of professional publications and presentations; job description listing duties and responsibilities; copies of diplomas, certificates and licenses; court testimony; research; participation in continuing education programs.

B-3 **Is the Director responsible for**

a) **E** **daily management of the laboratory?**

b) **E** **preparation and revision of the standard operating procedure manual?**

c) **E** **establishing procedures for validating new assays?**

d) **E** **maintaining a quality assurance program?**

e) **E** **training laboratory staff?**

The director has to be familiar with all aspects of the laboratory's operations. The director must be responsible for the management of the laboratory, and for the development of a complete, up-to-date procedure manual that is available to, and followed by, all personnel performing toxicological testing. The director must establish a procedure for validating new drug assays and will also be responsible for maintaining a quality assurance program to ensure the proper performance and reporting of all test results. The director must be responsible for (1) ensuring that the laboratory personnel are adequately trained and experienced to conduct the work of the laboratory and (2) maintaining the competency of laboratory personnel by

monitoring their work performance and verifying their skills. Authority can be delegated commensurate with assigned responsibilities.

B-4 **D** **Does the laboratory have qualified personnel who can substitute for the director in his/her absence?**

The range and type of duties of laboratory personnel will vary according to the size and the scope of the laboratory. It is desirable that laboratories should have a person who has sufficient training and experience to substitute for the director in case of his or her absence.

B-5 **E** **Are the personnel trained appropriately?**

Training and development of personnel is essential in order to increase productivity, improve performance and enable them to assume greater responsibilities. A training program to develop technical skills of an employee is important in each area of expertise. Personnel have to be familiar with all areas of toxicologic testing within their responsibility and understand how their responsibilities relate to the operation of the laboratory as a whole. Training should include, but not be limited to theory and practice of methods and procedures that the individual performs, understanding quality control practices and procedures, maintenance of chain of custody, laboratory safety, etc. The director is responsible for providing adequate training of personnel and for maintaining the competency of laboratory personnel by monitoring their work performance and verifying their skills. Records must be maintained to support the qualifications, experience and training for all personnel. These records may either be maintained in an individual's personnel file or in separate training files.

B-6 **I** **Is personnel experience and training documented?**

Training checklists/summaries
Resume
Copies of certificates
Copies of diplomas
Copies of licenses
Testimony experience
Other

B-7 **I** **For the laboratory personnel that testify, is their experience and training commensurate with the testimony expected?**

Many laboratory personnel rarely, if ever testify. When they do, it is frequently only to give factual testimony regarding the handling of evidence and performance of tests. Training for testimony may be limited, and given between receipt of the subpoena and the date the testimony is expected. However, the testimony of more senior toxicology personnel, especially those expected to give opinion evidence will need more extensive training. Formal training for more senior and experienced staff may be minimal, although experience should be documented as part of their curriculum vitae or training file.

B-8 **I** **Does the laboratory have sufficient technical personnel to handle the workload?**

If key quality issues are deficient and the laboratory director is claiming not to have sufficient time to attend to them because of staff shortages, then the answer to the question may be “no”.

B-9 **D** **Are job descriptions available for all technical staff in the laboratory?**

Job descriptions may be available in the individual “training files”, or they may be filed separately in the human resources area. The job description should be available somewhere, and the employee should normally have read it, and have easy access to it.

B-10 **D** **Does the laboratory have a written policy for the continuing education of technical personnel?**

Management of the laboratory should recognize the importance of the continued training of the technical staff, commensurate with their job function. Supervisory or lead technical personnel may require periodic specialist training, which may or may not be available from within the institution. The training of more junior technical personnel might typically be by supervisory personnel. Forensic toxicologists who testify or provide interpretation might be encouraged to review the forensic literature on a regular basis and at least periodically attend relevant local or other forensic conferences.

Section C: STANDARD OPERATING PROCEDURE MANUAL

C-1 **E** **Does the laboratory have a Standard Operating Procedure manual (SOP) which includes procedures for the routinely used analytical methodologies?**

Laboratories must have a complete and current Standard Operating Procedure (SOP) manual that describes all the routinely used analytical and administrative procedures. The SOP will be examined for completeness and to ensure it is current.

For routine analytes the manual should include the following: a) theory and principle of the procedure, b) instructions for preparation of reagents and controls, c) details of the analytical procedures, d) information about special handling of reagents and specimens, and e) references.

C-2 **E** **Does the SOP contain a list of analytes that are routinely quantitated?**

C-3 **E** **Is the list of routinely quantitated analytes consistent with the laboratory´s stated mission?**

The manual should include a list of the analytes commonly screened for and quantitated by the laboratory. This list should be compatible with the laboratory´s stated mission. It is essential that the laboratory has a mission statement of its goals. The goals of a medical examiner's laboratory would include assisting the medical examiner in determining the cause and manner of death through the analysis of post mortem specimens and through the interpretation of the analytical results, if necessary in a court of law. In this case the list of routine analytes should include alcohol, drugs of abuse, over the counter drugs, other therapeutic agents and toxic chemicals.

For a laboratory involved in human performance toxicology the mission statement would be different and reflect its goal of assisting law enforcement agencies in the detection of the "impaired driver". This goal would require the analysis of body fluids (primarily blood, serum or urine) and the interpretation of the results, if necessary, in a court of law. In this case the list of routine analytes should include those substances that may modify human performance or behavior.

C-4 **D** **Does the SOP contain guidelines for as to which specific tests are to be performed on different types of cases?**

C-5 **D** **Are these guidelines consistent with the laboratory's stated mission?**

The tests performed on a specific case should be sufficiently comprehensive to cover the common analytes which might reasonably have a bearing on the case. The *extent* of the testing may depend on the initial test results and history received. For example, if a known cocaine abuser is found dead and high concentrations of cocaine are found after preliminary analysis, it may not be important to proceed with other tests (obviously depending upon the potential for criminal or civil litigation). However, if for example, tests for morphine and cocaine were negative, it is reasonable to expect tests for less common drugs of abuse to be performed (e.g.

hydromorphone, fentanyl, amphetamine analogues). Similarly, toxicological analyses in the sudden death of an elderly person which remains unexplained after autopsy might reasonably include therapeutic drugs known to be prescribed to that person for natural disease (e.g. digoxin, beta-blockers).

C-6 **E** **Are the screening methods used appropriate for the laboratory's stated mission?**

The effectiveness of a toxicology laboratory in detecting drugs or other poisons will obviously depend on the nature and extent of the methods that comprise the initial "screen". For obvious reasons, it is neither possible nor desirable to specify a minimum group of tests that must be performed. Typically, though, it will be a combination of immunoassay and chromatographic tests. The judgment of the inspector is important in assessing the effectiveness of the screening tests performed. However, there are two considerations in answering this question. First, what is the mission of the laboratory and what does the client (e.g. police; pathologist) require. A "drug screen" may be inherently limited, but the client is aware of, and willing to accept those limitations. For example, for DUI work, some jurisdictions may only require an immunoassay screen for drugs of abuse with appropriate confirmation of "positives". The second consideration is whether the laboratory is conducting a "limited screen", but implying from the wording of the report that a reasonably comprehensive drug screen has been performed. This is a difficult, but important question that may require discussion with the laboratory director and careful review of the requests made to the laboratory and reports generated.

C-7 **E** **Has the laboratory Director reviewed, dated and signed each procedure?**

C-8 **D** **Has the laboratory Director or his/her designee dated and initialed all changes?**

C-9 **E** **Is there documented evidence of review of the SOP by the Director at least annually?**

It is desirable that the Director review, sign and date each change made to the SOP. These changes may result from suggestions of the laboratory staff or from modifications made to the procedure by the Director. The Director may delegate this responsibility to an individual with supervisory responsibility for the scientific aspects of the laboratory. If the Director has delegated this responsibility, there should still be evidence of review by the Director of the entire procedure, at a minimum annually. The review may be documented by signature on a summary sheet.

C-10 **I** **Is the laboratory SOP, or the appropriate sections of the SOP, available to the analysts in the laboratory?**

All laboratory staff should have easy access to the SOP. In some laboratories abbreviated SOPs will be available at the bench. These should be consistent with the approved manual.

C-11 **I** **If the laboratory uses abbreviated procedures (e.g. index cards) at the bench, are they in agreement with the SOP?**

C-12 **Does the SOP contain sections on:**

a) **E** **specimen receiving, accessioning, aliquoting and storage?**

b) **I** **procedures for recording the transfer of specimens?**

c) **I** **procedures for retention and disposal of specimens?**

d) **I** **procedures for the set-up and normal operation of instruments?**

e) **E** **copies of the routinely used analytical methodologies**

f) **E** **description of the quality assurance and quality control program?**

g) **E** **criteria for the acceptance of analytical data?**

h) **I** **protocols for recording, reviewing and reporting results?**

C-13 **E** **Do the assay protocols in the SOP contain sufficient detail to allow the analyst to perform the assay?**

The written procedures should contain sufficient detail to enable the routinely performed assays to be carried out without reference to supplementary information sheets or cards which are not part of the SOP.

C-14 **Where appropriate, does each of the analytical methods contain sections describing:**

a) **D** **the principle of each analytical procedure?**

b) **E** **details for the preparation of reagents, standards, calibrators and controls?**

c) **D** **references?**

C-15 **I** **Does the laboratory maintain copies of the outdated SOPs and the dates they were in effect?**

Copies of outdated SOPs are required to be kept so that the laboratory has an accurate record of the analytical procedures and analytical protocols that were in effect when particular results were generated, in case of legal challenge.

C-16 **I** **Is there a protocol for approving or handling deviations from written procedure?**

The unique nature of forensic work means that on occasion it may be necessary to deviate from or modify written procedures, in order to accommodate an unusual sample type or condition, multiple or unusual analytes, or small sample volume. The laboratory should have a protocol for documenting these and other deviations from normal practice.

Section D: SPECIMENS, SECURITY AND CHAIN OF CUSTODY

D-1 **D** **Does the laboratory issue instructions to user agencies, including the types and amounts of specimens required?**

The proper selection, collection, submission and storage of specimens for toxicologic analysis is important if analytical results are to be accurate and their subsequent interpretation scientifically sound. The laboratory should develop and provide detailed guidelines and instructions to all user agencies and parties the laboratory serves. These instructions should include recommendations regarding:

-types and minimum amounts of specimens needed for the analysis and subsequent interpretation
-specific requirements for the type and size of specimen containers
-type and amount of preservative to be added, if appropriate
-instructions for proper labeling of individual specimen containers
-acceptable conditions for packing and transportation
-instructions how to properly fill out all chain-of-custody documentation

D-2 **E** **Does the laboratory compare the information on the labels against that on the requisition and document any discrepancies?**

Specimens should be labeled with appropriate identifying information and accompanied by a request form containing the same information. The document can also serve as the external chain-of custody form. At the time of specimen receipt, it should be ensured that the information on the labels matches that on the request documentation. Any discrepancies should be documented, for example in case files or in separate log books.

D-3 **E** **Does the laboratory assign identification number(s) to the specimens received?**

The manner in which individual specimens are identified within a laboratory may vary. It is a common procedure for individual specimens to each be given a unique "accession number" upon receipt in the laboratory. Alternative procedures may be acceptable, providing that each individual container of specimen is uniquely identified in some way. For example, some medical examiner laboratories use the ME case number, plus a "specimen designator" (e.g. "Bl" for blood). This is acceptable providing that multiple specimens of the same type (e.g. multiple vials of blood from the same case) may each be uniquely identified.

D-4 **I** **Does the laboratory document the condition of the specimens?**

It is important to maintain a document regarding the condition of specimens at the time of receipt. This document may either be maintained in an individual case files or in separate log books and should contain information such as: deficiencies in the integrity of external packaging, integrity of seals, amount of specimen and degree of decomposition. Other deficiencies may include the unusual appearance of a specimen (e.g. "watery" blood, bloody urine etc.).

D-5 **E** **Is entry to the laboratory controlled during working hours?**

Access to the forensic toxicology laboratory must be limited to authorized personnel. Unauthorized persons should be escorted and must sign a logbook upon entry and departure, indicating the time, date and purpose of the visit. The physical layout of the laboratory must be such that unauthorized persons cannot enter without detection. All exterior ingress/egress points require proper locks. All keys must be accounted for and their distribution limited.

D-6 **E** **Is the laboratory secure during non-working hours?**

The laboratory should be secured by locks during non-working hours to prevent unauthorized access. Additional security precautions may sometimes include monitoring devices (e.g. motion detectors) and security personnel in the building where the laboratory is located.

D-7 **E** **Does the laboratory secure short and long term specimen storage areas when not in use?**

Specimens must be stored in a secure manner at all times to maintain integrity. Proper security can be achieved by storing specimens in locked cabinets, refrigerators or rooms. It is acceptable to leave storage rooms unlocked when authorized personnel are present.

D-8 **I** **Does the laboratory have adequate space for short and long-term storage of biological specimens?**

Adequate and appropriate space should exist for short and long-term storage of biological specimens and other evidence.

D-9 **I** **Does the laboratory secure record storage areas?**

Records have the same evidentiary importance as the specimens and therefore must be stored in a secure manner. Records can be stored in a secured room, area, or file cabinet. Access should be restricted to authorized personnel (e.g. personnel assigned to records management, appropriate supervisory and laboratory personnel). "In use" records (e.g. incomplete files or those pending reporting or filing may be, as a matter of convenience, temporarily stored at different locations prior to final disposition. Temporary storage of such files outside of a locked cabinet or storage room is acceptable, providing the laboratory is secured and access is controlled by authorized laboratory personnel.

D-10 **I** **Does the laboratory maintain the available external chain of custody, requisition and/or shipping information?**

D-11 **I** **Does the laboratory document all persons handling the specimens?**

Transfer and handling of specimens should be clearly documented as part of the permanent laboratory records and should indicate, at a minimum, the date and identity of the individuals involved in the specimen transfer, and laboratory identification number. This document may be a logbook, worksheet or other suitable means of recording the information and does not necessarily have to be a strict chronological "z-style" chain of custody document. Batch forms are acceptable if transfer involves multiple specimens.

D-12 **I** **Are specimens stored in such a manner as to, as far as practical, preserve them in the same condition they were received?**

Specimens received in the laboratory should, as appropriate, be refrigerated as soon as possible after arrival, to preserve them in the condition in which they were received. If it is known that the specimens cannot be analyzed for an extended period (e.g. several weeks), the samples should be frozen. Similarly, samples that have been analyzed which are expected to be the subject of litigation should be frozen until required for reanalysis or disposal.

Section E: QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance encompasses all aspects of the analytical process, from specimen collection and receiving through analysis, data review and reporting of results. A quality assurance program should obviously include a quality control program that is used to evaluate whether the analysis is operating within defined tolerance limits. An effective program should detect both random and systematic errors in a timely manner and allow the laboratory to take corrective action.

To quote from the Guidelines:

> "Quality assurance assumes a unique role in the forensic science disciplines because results are subject to challenge in the "adversarial" justice system."

The components of a QA/QC program include proper quality control specimens, the use of validated testing procedures, timely evaluation of QC specimen results, and implementation and documentation of corrective action.

Because there are variations in QC programs and the understanding of the terms used, the Guidelines defined standards, calibrators and controls as follows:

Standard: a reference material possessing one or more properties that are sufficiently well established that calibrators can be prepared.

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

Controls: 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 specimens. Controls from all three 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.

It is essential that the laboratory implements and continues to practice quality control, and that its implementation, review and any necessary corrective action is documented. This documentation must be maintained for the same period as case records.

E-1 **E** Is a suitably qualified individual assigned day-to-day responsibility for QA/QC?

A specific individual must be assigned to the day-to-day responsibility for QA/QC. In a smaller laboratory that individual might be the laboratory director. However, in most laboratories, although the director will retain overall responsibility for QA/QC, day-to-day responsibility will be delegated to a deputy, supervisor or other responsible technical person. Suitability should be judged in the context of academic qualifications, experience, knowledge and job function, but does not necessarily require formal training in QA/QC.

E-2 **I** **Is the QA/QC program of the laboratory reviewed annually?**

It is required that the QA/QC program as a whole be reviewed at least annually, to ensure that it is up-to-date and is effective. That review will normally be documented as a signed and dated review (or revision) of the QA/QC section of the laboratory's SOP. It should be noted that the annual review is of the program as a whole, and does not just apply to QA/QC data, which must be reviewed on a considerably more frequent basis. In particular, the review should address whether proper corrective action has been taken where deficiencies exist.

E-3 **E** **For *qualitative* assays does the laboratory include appropriate positive and negative controls with each batch of specimens for analysis?**

With each analytical run of specimens, whether a single specimen or a batch, and whether qualitative or quantitative, controls should be carried through the procedure with the unknowns. It is recommended that each batch of specimens include at least 10% controls, including at a minimum, one positive and one negative.

Although there is no need to monitor the concentration of a positive control in a qualitative assay, the target concentration must challenge the assay. For example, it would be unacceptable to select a positive control for a benzoylecgonine immunoassay at 4000 ng/mL if the decision point is 300 ng/mL; an acceptable concentration might be 400 ng/mL. On the other hand, for a chromatographic assay, the concentration should be such that it is routinely detectable by the chromatographic system.

For some forensic toxicology procedures, providing a true control is no more difficult than for any other test. Fortifying analyte free matrices such as tissue homogenates, expired blood bank blood or plasma to simulate the unknown specimen is acceptable. For others, the matrix may be unique (e.g. decomposed tissues, bone, hair or nails) and providing a true control is very difficult. In such cases the laboratory should select the most appropriate control, for example when hair is analyzed a control prepared in the digestion medium would be acceptable.

Open controls (controls whose identity and concentration is known to the analyst) can be purchased commercially, prepared in the laboratory or saved and pooled from previous cases. Regardless of the source, the concentration of the analyte in frequently run controls must be validated. There is no requirement to analyze blind controls (controls whose identity is unknown to the analyst), however it is recommended that for laboratories routinely performing analysis in batches, blind controls be included to verify the aliquoting process.

E-4 **E** **For qualitative assays, are the results of these controls available for review?**

Control results must be recorded as part of the analytical record. For a qualitative assay the control is used to monitor the ability to discriminate negative specimens from positive ones. Therefore it there is no need to monitor concentration. If the laboratory does so, for frequently run assays, it should plot the results using one of a variety of techniques. Typically Levy-Jennings charts are used. Some laboratories may use cumulative sum (cusum) charts or mean/range charts.

E-5 **E** **Does the laboratory have written criteria for the acceptance of the qualitative controls?**

Criteria used by the laboratory must be included in the SOP.

E-6 **I** **Are these criteria appropriate?**

Where the screening test is qualitative, it is acceptable to indicate whether the positive control tests positive and whether the negative control tests negative. If quantitative results are determined, acceptable results are those whose determined concentration are within the mean plus or minus a valid statistical measure, for example two standard deviations or ±20%.

E-7 **E** **For *quantitative* assays of common analytes, does the laboratory include appropriate controls with each batch of specimens for analysis?**

For quantitative assays, controls are used to verify the calibration and to monitor its stability. In this case the controls used should include, at a minimum, a negative and a positive control that realistically monitors the performance of the assay. Additional controls may be necessary to challenge the linearity of the procedure and ensure reliable quantitative values along the entire curve. The number and concentrations of acceptable controls will be determined by the extent and nature of the calibration and method validation, and the extent to which case results are reported quantitatively outside the range of the (multi-point) calibration. Even for multi-point calibrations, determination of an "acceptable" calibration coefficient does not guarantee linearity at the extremes of a calibration curve, unless quantitative acceptance criteria are applied to each calibrator. For a single point calibration, it is important that at least two positive controls be run, which validate linearity of the calibration within the reporting range. Where linearity is determined as part of method validation, that does not guarantee linearity at the time the assay is run with case samples.

It is also important for multi-analyte assays, that each analyte be associated with a control which contains the same analyte, or at a minimum one with similar chemical properties. For example, a control containing amitriptyline might be used to verify other tertiary amine TCAs, whereas nortriptyline might be used for other secondary amine TCAs.

E-8 **E** **Are the results of these controls available for review?**

For frequently performed quantitative assays the laboratory should plot the results using one of a variety of techniques. Most commonly Levy-Jennings charts will be used. Some laboratories may use cumulative sum (cusum) charts or mean/range charts. Acceptable results are those where the determined concentrations are within the mean plus or minus a valid statistical measure, for example two standard deviations or ±20%.

E-9 **E** **For quantitative controls, does the laboratory have written criteria for their acceptance?**

Whatever criteria are used by the laboratory must be included in the SOP.

E-10 **I** **Are these criteria appropriate?**

The appropriateness of acceptable criteria is to some extent based on the assay. Obviously, if the laboratory uses a range of two standard deviations for all quantitative assays this is an accepted, and acceptable practice, providing that the absolute deviation from target is not unreasonable (e.g. ±30 - 40% would normally be considered unacceptable). Other acceptable criteria include use of the mean or target value ±20%. Whatever criteria are used, the laboratory should clearly demonstrate that they are appropriate for the assay by, where practical, monitoring the precision of frequently performed assays over time. It may sometimes be appropriate to set less stringent quantitative criteria for a control which is close to the LOQ of the assay, compared with a mid-range control, especially where concentrations approaching the LOQ are of little toxicological significance.

E-11 **E** **If the laboratory prepares its own calibrators and controls, are these made using independently prepared stock drug solutions?**

Calibrators and controls should be independently prepared from a separate weighing or initial dilution or obtained/derived from other sources. If both the calibrator and control(s) are derived from the same source, the laboratory may introduce an undetectable bias into its results, since controls are used to verify the calibration.

E-12 **I** **Are control results reviewed by a qualified individual, and if so how frequently?**

Signing and dating the QC record constitutes evidence of review. In some cases the Director may designate this review to a laboratory manager or quality control supervisor, but even in this situation he/she should review the data on a periodic basis.

E-13 **D** **Does the laboratory independently verify reference materials?**
Generally, high quality reference materials and stock solutions obtained from a supplier are provided with a certificate that describes the identity, quality and concentration of the material. The laboratory should verify this information. The verification may involve obtaining a full spectrum GC/MS analysis with comparison to library spectra and absence of additional/ interfering chromatographic peaks, measurement of a physical constant (such as, melting point, refractive index), or use other analytical techniques (such as HPLC, IR, UV/VIS). This verification may need to be repeated if the reference material is used beyond its expiration date.

E-14 **E** **Does the laboratory take corrective action when control results exceed specified limits?**

It is important that the laboratory recognize when results for a control are unacceptable. In this case, the laboratory should have a procedure in its SOP to describe the corrective action and how it will be documented. For example the failure of a positive control in a qualitative assay may require the laboratory to retest the entire batch or only particular specimens.

E-15 **E** **Is this corrective action documented?**

Corrective action may be documented as an annotation on the analytical record, as a separate memorandum attached to the data, or referenced to a separately filed package of material.

E-16 **I** **Was the corrective action appropriate?**

The appropriateness of the corrective action is dependent on the assay. For qualitative immunoassays it may be necessary to repeat all specimens in a batch, for example if the negative control tests positive, or to repeat only those that are "positive", for example if the positive controls tests negative. On the other hand, in quantitative assays the SOP should clearly define the acceptance criteria for controls and what corrective action to be taken if they fail. Only a review of the analytical data will show if the laboratory is following these criteria.

E-17 **E** **Are proficiency test results reviewed by the laboratory director?**

At a minimum the director should review and sign-off on all proficiency test results, and where necessary ensure that appropriate corrective action is taken.

E-18 **E** **If unacceptable results occurred in PT programs did the laboratory take corrective action?**

E-19 **E** **Was the corrective action for unacceptable PT results appropriate?**

If the proficiency test program to which a laboratory subscribes has criteria for acceptable performance, these shall be used. If the laboratory fails to satisfy the program´s acceptance criteria, then there should be documentation of review and corrective action. Such corrective action may include repeat analysis of the proficiency testing specimen, preparation of new calibrators or a complete re-validation of the analytical procedure. If the laboratory reports a false positive or false negative, it is important that this review, and if necessary corrective action, was taken promptly.

If criteria for acceptable performance do not exist, the laboratory director should make his or her own decision as to whether performance has been satisfactory, where practical based on the following: no false positives; ethanol within ±2 S.D. of the participant mean or ± 10% weighed-in target; for drugs, the challenges should be within ±2 S.D. of the participant mean or ± 20% weighed-in target for drugs. Corrective action or investigation (if only limited to an audit of the raw data) is sometimes appropriate, even if the results are within ±2 S.D. For example, the proficiency test S.D. range for some analytes is so large that ±2 S.D. can represent from near zero to at least double the weighed-in target or participant mean.

False positive results require the most rigorous investigation. Extensive and thorough investigation is expected. However, the error may be considered less serious if it is clerical in nature and unique to the way results are reported for the particular PT program (e.g. use of an incorrect analyte code). The extent of investigation and corrective action required for a false negative will depend of whether the analyte might ordinarily be expected to be detected by the laboratory at the spiked concentration, or whether detection is judged to be unimportant for the mission of the laboratory.

E-20 **D** **Does the laboratory properly label reagents as to identity (essential), date of preparation, expiration date and identity of the preparer?**

It is good laboratory practice for the date of preparation, expiration date and identity of the preparer to be included on the label of in-house prepared reagents. For purchased reagents, the date received and/or opened should be labeled.

E-21 **D** **Does the laboratory validate new or freshly prepared reagents?**

There are two primary ways to check new reagents before using them. First, a laboratory can prepare separate validation batches containing only controls prepared with the new and current reagents. Second, a laboratory can prepare routine batches of specimens, including controls, with the new reagents and compare the results of controls from preceding batches, prepared with the current reagents.

The most critical reagents requiring such checks are:

- immunoassay kits
- derivatizing reagents
- organic solvents and mixtures for chromatography and extraction
- pH-specific reagents and buffers
- hydrolysis reagents
- solid phase extraction reagents
- TLC plates and spray reagents

E-22 **D** **Does the laboratory periodically check the accuracy of pipettes which use disposable tips.**

The laboratory should periodically check the accuracy of pipettes used for quantitative measurements. Typically gravimetric or colorimetric methods are used. Where a pipette is solely intended to qualitatively dispense reagents, it should be labeled as such (e.g. “qualitative only”).

E-23 **D** **Does the laboratory have a preventive maintenance schedule and service logs for all instruments in routine use? (Indicate X if deficient):**

Spectrophotometer(s) _____
Gas chromatograph(s) _____
High performance liquid chromatograph(s) _____
Gas Chromatograph-mass spectrometer(s) _____

Most instruments require some type of routine maintenance. This can usually be divided into routine service that the operator performs (e.g. for GC, liner and septum changing, cutting columns etc), service that is performed less frequently (e.g. changing rough pump oil; MS source cleaning), in addition to ad hoc work performed by qualified service personnel. Records of scheduled service may be included as an integral part of the service log, or as part of a separate maintenance schedule for the laboratory, such that it is readily evident to users of the equipment and QA staff. However, all service work should be documented and that documentation readily available to operators of the equipment.

E-24 **D** **Are the records of the preventive maintenance, and other maintenance available to the staff operating the instruments?**

Records of service or maintenance should be accessible near the instrument it pertains to, or failing that, in a location known and readily accessible to the operator of the instrument.

E-25 **D** **Where appropriate is equipment which is uncalibrated, broken or otherwise out of service clearly marked as such?**

It is a requirement that equipment that is broken or out-of-service pending calibration be clearly marked as such unless it is such a condition or location that its status is obvious. For example, adjustable pipettes that require cleaning or recalibration should not be left such that they are apparently available for use. Larger equipment, such as centrifuges, should be clearly tagged as out-of-service if they are unsafe or require maintenance (e.g. due to fractured, corroded or unbalanced rotors of a centrifuge).

E-26 **D** **Does the laboratory monitor, and as necessary, record temperatures regularly on all equipment where temperature control is necessary, such as water baths, heating blocks, incubators, ovens, refrigerators, and freezers? (Indicate X if deficient)**

Water bath(s)	_____
Heating block(s)	_____
Incubator(s) and oven(s)	_____
Refrigerator(s)	_____
Freezer(s)	_____
Refrigerated centrifuge(s)	_____
Other	_____

For some equipment (e.g. refrigerators, freezers) it is important to regularly monitor and to record critical temperatures (for example where specimens are stored). At a minimum, temperatures and corrective action should be record where set limits have been exceeded. For some devices (e.g. heating blocks, water baths), temperature may be monitored and adjusted at the time of use.

E-27 **D** **Does the laboratory have the analytical balances cleaned, serviced and checked periodically by qualified service personnel?**

E-28 **D** **Does the laboratory have standard weights (NBS Class S or equivalent) available for checking accuracy?**

E-29 **D** **Have in-house computer programs, spreadsheets and macros which are used to calculate or report analytical results been adequately validated?**

In-house computer programs, spreadsheets or macros used to calculate or report analytical results should be validated prior to routine use and periodically checked thereafter (e.g. annually, or as appropriate for intended use). Backup copies of validated files should be kept secure from general use (e.g. physically secure, via password protection or read-only status). Spreadsheets in particular can easily have formulas in cells changed without it necessarily being obvious to the user. The extent of monitoring some macros or programs may simply be

to ensure that it appears to do what it was written for, without any special checks (e.g. draw a set of 3 overlaid chromatograms). Validation of commercial software is not required.

ANALYTICAL PROCEDURES

Section F: IMMUNOASSAYS

A number of immunoassays are used to screen for drugs and their metabolites, particularly the drugs of abuse. These immunoassays include radioimmunoassay (RIA), enzyme immunoassay (EIA), kinetic interaction of micro particles in solution (KIMS), fluorescence polarization immunoassay (FPIA), and enzyme linked immunosorbent assay (ELISA).

Although these applications are suitable for the screening of urine specimens by commercial drug testing laboratories, they may not be of much use to the forensic toxicologist. Very often he/she needs to screen specimens other than urine and to use different cut-offs. It is certainly acceptable for them to do so provided that the modified assay has been validated by the laboratory and that this validation has been documented.

As with all qualitative assays it is necessary to analyze negative and positive controls with each batch of specimens. Positive controls should be targeted at a realistic concentration that appropriately challenges the assay (e.g. 125 - 200% of the cut-off). Negative controls may be spiked with analyte at an appropriate concentration below the cut-off (e.g. 50 - 80%), or may contain no analyte. The actual concentrations, frequency and position of controls will depend on the matrix and application. In many laboratories, immunoassay results are individually reported, and therefore accuracy and precision around the cut-off is important. However if the immunoassay is only being used as a guide to whether further testing is performed (e.g. GC/MS for benzodiazepines), then use of positive and negative controls *that closely bracket the cutoff* is less important.

F-1 **I** **Is the laboratory's instrumentation maintained and serviced regularly, according to the manufacturer's recommended protocol?**

In addition to containing instrument specifications and routine testing procedures, the instrument operator's manual contains recommended maintenance procedures to be performed daily, weekly, monthly, etc.; troubleshooting diagrams or flow charts and directions for equipment servicing that can be performed by the operator. Many operator manuals contain service log sheets and maintenance checklists that can be copied and used in the laboratory. Proper maintenance will increase the life span of the instrument and decrease the likelihood of instrument malfunction. These records should be readily available to the operator of the instrument and supervisory personnel responsible for data review. They are indicators that the instrument is operating properly. Changes in instrument and reagent performance with time can be noted.

F-2 **D** **Are the maintenance records readily available to the technical staff operating the equipment?**

F-3 **E** **If the immunoassay is being used to test specimens for which the assay is not approved by the manufacturer, or if the test method recommended by the kit manufacturer has been modified, has the laboratory validated these changes?**

It is necessary for the laboratory to validate any modification to a commercially available immunoassay. There are three common situations under which this may occur. Following are suggested validation protocols:

1) Use of a different specimen from that recommended: the most common example of this situation is the analysis of blood rather than urine. In this case the laboratory has to document that there is adequate separation between negative specimens and the cut-off.

2) Use of a different cut-off from that recommended: the most common example of this situation is the lowering of the cut-off to detect any drug present. In this case the laboratory has to document that there remains adequate separation between a negative specimen and a positive one. If this is the case the laboratory should also document that there is adequate separation between negative specimens and the cut-off. For laboratories that have lowered the manufacturer's cut-offs, it is recommended that the response of the negative plus two standard deviations does not overlap the response of the cut-off.

3) Modification of the reagents: similar validation studies to those described for 1 and 2 are necessary, but in addition it may be necessary to confirm the cross-reactivities quoted by the manufacturer, particularly if the laboratory is using the kit to detect a drug grouping, for example the benzodiazepines. For qualitative assays it is required that the laboratory analyze replicate calibrators at the cut-off of the assay. The best example of the use of replicate calibrators is the calibration of an immunoassay using a cut-off calibrator. The SOP should clearly define the number of calibrators to be used, the criteria for acceptance of the calibrators and corrective action to be taken. At the very least the laboratory should run the calibrators in duplicate and take the mean as the cut-off reading.

To verify that the immunoassay retains its specificity the laboratory should analyze a randomly selected set of specimens and confirm all immunoassay positives by a reference procedure, such as GC/MS.

F-4 **I** **If the laboratory analyzes specimens other than those which the manufacturer recommends does it analyze matrix matched controls?**

This situation is most commonly encountered when the laboratory is using an immunoassay designed for urine and applying it to blood. In this case the laboratory should use calibrators and/or controls in a similar matrix. If the assay is being used for quantitation, it is also necessary that the concentrations of drugs in the controls have been verified by a reference procedure.

F-5 **I** **Does the laboratory have protocols to account for potential contamination and/or carry-over if it uses automatic pipetting/diluting equipment?**

Very often the larger laboratories will use automatic pipetting/diluting equipment or automated analyzers (such as the Hitachi 717 or the Olympus AU800). Because these instruments are used to analyze specimens that can contain large concentrations of analyte, it is important that the laboratory has validated this potential for carryover and taken corrective action if it occurs. An example of appropriate corrective action is reanalyzing consecutive positives with a negative control between them, when the first positive urine has a higher concentration than the carryover limit.

F-6 **D** **If the laboratory uses radioimmunoassay, does it determine background counts before each run or daily, including the background in each well of a multi-well counter?**

For RIA analyses, counting efficiency and statistical biases should be determined on a regular basis and background counts should be determined on a daily basis or whenever the testing is performed. These values should be entered in a record book to monitor changes in instrument performance.

Section G: CHROMATOGRAPHY, MASS SPECTROMETRY AND SPECTROPHOTOMETRY - GENERAL QUESTIONS:

G-1 **E** **Are the analytical protocols used for the chromatography based assays appropriate?**

The protocols used should be sufficiently comprehensive to satisfy the mission of the laboratory. For example, it would be inappropriate for a laboratory to indicate they performed "drug screening" on a medical examiner case when testing was only based on immunoassay, even if any positive findings were subsequently confirmed by mass spectrometry (unless the report included a statement indicating the limitations of the screen). The laboratory should also have the capability to quantitate an adequate range of drugs in blood and other specimens with an acceptable degree of accuracy and precision, sufficient for the mission of the laboratory, recognizing that some tests may have to be sent out to a reference laboratory.

G-2 **I** **Does the laboratory prepare quantitative calibrators and controls using an appropriate matrix?**

Spectrophotometry	_____
Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

It is important that an appropriate matrix be used, since for some analytes recovery is very matrix-dependent. For some forensic toxicology procedures, providing an appropriate matrix is no more difficult than for any other test. Typical examples include tissue homogenates, expired blood bank blood or plasma or urine. For others the matrix may be unique (e.g. decomposed tissues, bone, hair or nails) and providing a matrix blank can be very difficult. In such cases the laboratory should select the one that is closest to the specimen being analyzed. For example when hair is analyzed the digestion medium would be acceptable as a matrix blank.

It may be acceptable to analyze specimens of differing matrix against a common calibration, if matrix-matched controls are run at the same time, or if it has been demonstrated using the same or a similar analyte, that the assay is not significantly matrix-sensitive. For example, it may be acceptable to analyze a serum specimen for lorazepam against a blood-based calibration, if it had already been shown that there was relatively little difference between the results obtained for diazepam (for example) in blood versus serum in the same or a similar assay.

G-3 **E** **For qualitative and quantitative assays, does the laboratory analyze calibrators and/or controls with each batch of specimens?**

Spectrophotometry	_____
Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

Comments:

Case specimens should never be assayed in isolation. At least one calibrator or control should be processed at the same time, in order to ensure the veracity of the extraction process. For example, to ensure that if the sample tested negative, a calibrator or control should be extracted and run simultaneously to demonstrate that there were no analytical deficiencies. See the question relating to "historical" calibrations.

G-4 **E** **Does the laboratory analyze calibrators and controls in the same manner as unknowns?**

Spectrophotometry	_____
Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

All calibrators and controls have to be analyzed in the same manner as the specimens, where this is possible. One of the most common faults of a laboratory is that calibrators and controls are not subject to hydrolysis, or some other pre-treatment.

G-5 **E** **Does the laboratory establish a valid calibration for each quantitative assay?**

Spectrophotometry	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

Linearity of the procedure should be established by using at least three positive calibrators. The concentration of the calibrators should be such that they bracket the anticipated concentration of the specimens. 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 and the specimen re-extracted, or the analyte reported as being less than the value of the lowest calibrator. A matrix blank or negative control should also be run to ensure that no significant analyte signal is contributed by the reagents or sample

matrix.

For assays that are performed routinely, it is acceptable for the laboratory to perform a single point calibration, as long as controls are run that bracket the expected concentration range of the analyte. In this case the laboratory must document the linearity of the assay over the expected concentration and validate this linearity periodically. This validation should occur at least every six months.

G-6 **E** **If the laboratory uses historical calibration for an assay, are calibrators and/or controls run with each batch of specimens for analysis to check stability of the calibration?**

Spectrophotometry	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

It is acceptable for laboratories to use historical calibration curves if they have demonstrated and documented the linearity and precision of the curve over time. If they do use historical curves, then they must validate the calibration by using controls with each batch of specimens. These controls should be such that they validate the calibration over the entire range of the curve. Although, it is better to use controls for this purpose, it is acceptable for the laboratory to validate the calibration by analyzing calibrators as long as these cover the entire range.

G-7 **E** **Are appropriate criteria established for the acceptability of calibration data?**

Spectrophotometry	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

There are a variety of procedures for establishing the acceptability of calibration data, and these are often listed as options within data reduction software included with modern analytical instruments. Most commonly, laboratories use linear regression and define acceptability based on the “r” value. Typically, values of greater than 0.98 are acceptable with non-deuterated internal standards and, for mass spectrometry, greater than 0.99 with deuterated internal standards. For frequently run assays, it may be valuable to monitor the slope of the calibration line. A significant deviation from the historical slope may indicate a problem with the assay.

Other procedures include back calculating the concentrations of calibrators based on the response factor of the previous calibrator(s) within that run, selection of the best curve fit (not necessarily a linear response) and use of first or second derivatives (for spectrophotometry). Regardless of which the laboratory uses, the SOP should clearly indicate which procedure is used for which analyte and the criteria for acceptance.

If the laboratory uses more than three calibration points, the SOP must clearly indicate how many points can be deleted and under what circumstances. The SOP should also address

which results can be reported after calibrators are deleted.

G-8 **I** **Does the laboratory use an internal standard for qualitative analysis?**

Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

It is recommended that laboratories use internal standards for chromatographic assays that are used qualitatively. By doing so the laboratory can monitor recovery of the batch and also determine whether a dilution is necessary for the quantitative assay. An internal standard will also assist in identifying the unknown analyte, if the laboratory uses relative retention times for this purpose.

G-9 **E** **Does the laboratory use an appropriate internal standard for quantitative analysis?**

Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

The laboratory must use an internal standard for quantitation using chromatographic procedures. It is not acceptable for the laboratory to add a "marker" after the extraction (and derivatization) are completed. This is regarded as an external standard.

Ideally the internal standard should have chemical and physical properties as similar to the analyte as possible. If the analyte is to be derivatized, the internal standard should form an analogous derivative. For GC/MS assays deuterated internal standards are usually preferred where available.

G-10 **E** **For qualitative and quantitative assays, does the laboratory check for carry-over and contamination?**

Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

Laboratories often use automated injection systems. Because these instruments are used to analyze specimens that can contain large concentrations of analyte, it is important that the laboratory has validated this potential for carryover and taken corrective action if it occurs. Possible sources of contamination include homogenizers, inadequately cleaned glassware (e.g. micro-vials) and transfer pipettes. Appropriate corrective action might include analyzing solvent blanks between specimens.

Detection of carry-over or contamination may sometimes require a careful review of the analytical results against the case history, and the reanalysis of specimens, or analysis of

multiple specimens. Where a laboratory routinely quantifies analytes in separate assays from that used to detect the substance, carry-over or contamination (within the laboratory) may be easy to detect. However, extreme caution is warranted where a drug is simultaneously detected and quantitated in a single specimen analyzed in a single assay.

G-11 **I** **Does the laboratory validate frequently used assays before implementation?**

Spectrophotometry	_____
Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

It is important that the laboratory validates each frequently used procedure before it is implemented. This validation data will depend on the assay and its application. For qualitative assays it should include data on specificity, potential interference and sensitivity. For quantitative assays it should include linearity, sensitivity, accuracy and precision. It is desirable that the method is compared with existing methodology or used to analyze certified reference material (if available).

Infrequently run quantitative assays may be regarded as "self-validating" if sufficient calibrators and controls are routinely run to demonstrate linearity, precision and sensitivity. For example, if a multi-point matrix-matched calibration is run, if each calibrator when read against the graph is acceptable (e.g. ±20%), and case results are only to be reported out within the calibrator range, and if an independently prepared control is run and acceptable (e.g. ±20% of target), the assay may be regarded as "valid".

G-12 **E** **Are validation records maintained?**

Validation data should be retained for at least as long as the assay is in-use. Validation records should be recorded *and summarized* in such a manner that they can readily be reviewed and understood by another toxicologist without reference to the individual who performed the validation. The validation package should clearly summarize what was done, what results were obtained, and what the conclusions were. Laboratories will not be unduly penalized for failure to have available documentation of validation that occurred prior to their initial accreditation. However, the ABFT Accreditation Program reserves the right to request validation of assays, or proof thereof, that are deemed critical to the mission of the laboratory.

G-13 **E** **Does the laboratory maintain records of testing data including laboratory accession numbers, specimen type, analyst and date of analysis?**

Spectrophotometry	_____
Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

Before results are reported, each batch of analytical data shall be reviewed by scientific personnel with experience in the analytical procedures used in the laboratory. At a minimum this should include: chain of custody documentation, analytical data and calculations, and quality control data. This data should be retained in a manner that it can be easily retrieved if requested.

G-14 **E** **Are the criteria for designating qualitative results as positive appropriate?**

Spectrophotometry	_____
Thin layer chromatography	_____
Gas chromatography	_____
High performance liquid chromatography	_____
Gas chromatography-mass spectrometry	_____
Liquid chromatography-mass spectrometry	_____

The SOP should clearly define the criteria for designating and reporting a positive analytical result. Definition of a positive analytical result by chromatography may be based on Rf (thin layer chromatography), retention index (gas chromatography), retention volume (high performance liquid chromatography), or retention time (gas chromatography and high performance liquid chromatography). For spectrophotometry or mass spectrometry it may be based on comparison with reference library data and a statistically based "fit". In gas chromatography-mass spectrometry it may be based on a combination of retention time and "fit", or if selected ion monitoring is used a comparison of ion ratios with those in the calibrator. If identification is based on selected ion monitoring, the ions monitored should collectively be characteristic of that analyte, not of a drug class. For example monitoring of m/z 58 alone indicates the presence of a dimethylamine fragment and is not characteristic of a particular compound.

Definition of a "positive identification" may require a combination of positive analytical results, for example a positive immunoassay and a positive gas chromatographic result. It is important that the laboratory recognizes the distinction between a positive analytical result and a positive specimen, reporting of a positive specimen based on a single positive analytical result is generally unacceptable.

G-15 **E** **Where practical, are all reported positives confirmed by a technique based on a different chemical principle from the first test?**

As a general matter of scientific and forensic principle, the detection of drugs and other toxins should be confirmed whenever possible by a second technique based on a different chemical principle. Generally the confirmatory (second) test should be more specific and sensitive than the first test. Wherever possible and practical, the use of mass spectrometry is recommended. 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-5).

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 that 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. However, in some circumstances a second, more specific immunoassay may be used to aid identification (e.g. use of a monoclonal morphine assay to better characterize a positive polyclonal opiate assay). In that example, chromatographic or MS confirmation of analyte identify would still be required.

Confirmation of the identity of an analyte in a different specimen from that used for the first test (e.g. urine and blood) is acceptable, as is confirmation in a second aliquot of the same specimen. However, confirmation of a drug or toxicant in the same original extract would not normally be regarded as acceptable, since that would not rule out the possibility that the vial or extraction tube used was contaminated.

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 or HPLC quantitation of a drug detected by immunoassay).

Notwithstanding the above, it is recognized that in some circumstances a suitable second test procedure is not available, or is unnecessary. For example, the probability that a 75% carboxyhemoglobin result obtained by a properly conducted spectrophotometric assay is incorrect in a well documented suicide is exceedingly low, whereas the unexpected finding of a 30% carboxyhemoglobin by a similar determination in blood from a motor vehicle accident victim holds a lower degree of certainty. However, at the very least the presence of a drug or toxicant should 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. Perhaps arguably, use of a second test for ethanol is not always necessary, especially where multiple specimens from the same person or case are analyzed, and where these results corroborate one another.

Although discouraged, reporting of immunoassay results for certain “therapeutic drugs” is acceptable if (a) the assay has been validated for the matrix being analyzed, (b) the result has little or no “forensic significance”, (c) the method of analysis is reported. Examples might include reporting a blood phenytoin concentration determined by FPIA in an epileptic known to be prescribed the drug, or in a similar situation, an asthmatic prescribed theophylline. However, if the drug has forensic significance (e.g. phenytoin toxicity or overdose), the analyte must be analyzed by a more forensically acceptable method (e.g. HPLC).

Nonetheless, 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).

Section H: SPECTROPHOTOMETRY (Includes CO-Oximeters)

H-1 **I** **Are the laboratory's spectrophotometers appropriately calibrated and maintained?**

In addition to containing instrument specifications and suggested testing procedures, the instrument operator's manual contains recommended maintenance procedures to be performed daily, weekly, monthly, etc.; troubleshooting diagrams or flow charts and directions for servicing the equipment that can be performed by the operator. Many operator manuals contain service log sheets and maintenance checklists that can be copied and used in the laboratory. Proper maintenance will increase the life span of the instrument and decrease the likelihood of instrument malfunction. These records should be readily available to the operator of the instrument and supervisory personnel responsible for data review. They are indicators that the instrument is operating properly. Changes in instrument and cuvette performance with time can be noted.

H-2 **D** **Are the maintenance records readily available to the technical staff operating the equipment?**

H-3 **D** **Are the laboratory's cuvettes in good optical condition?**

In all spectrophotometers, spectral resolution is proportional to light energy. The ability of the system to transmit light energy through windows, cuvettes and pellets depends upon their freedom from scratches, deposits, moisture, etc.

Section I: THIN LAYER CHROMATOGRAPHY

I-1 **I** **Does the laboratory have a written procedure for the preparation of mixed solvent systems, spray reagents and designation of lifetime?**

It should be indicated if eluting solvents are prepared by adding absolute volumes of solvents to each other or volume/volume preparations are used. The sequence with which eluting solvent components are added to each other may be important. The components of mixed solvent systems will have different vapor pressures and therefore will evaporate at different rates altering the composition of the solvent system. Some polar solvent systems will absorb water. The procedure used for eluting solvent equilibration in the developing tank (filter paper, TLC plate in the tank or not in the tank, etc.) must be standardized if reproducible results are desired.
Spray reagents will oxidize, absorb water, and decompose with time. It may be necessary to replace spray reagents at regular intervals whether they have been used or not. Refrigeration and/or storage in dark bottles may lengthen the useful life of spray reagents.

I-2 **D** **Does the laboratory have a written procedure for the storage of unused TLC plates?**

Most laboratory atmospheres have seasonal as well as daily variations in humidity. TLC plates will adsorb moisture and CO_2 if left out in the laboratory atmosphere. These may alter Rf values. Oven activation and storage in a desiccator may be necessary if the laboratory's atmosphere is quite variable.

I-3 **E** **Are standards applied to each thin layer chromatographic plate?**

As a mixed solvent system is used, the solvent remaining in the tank changes composition and the separating capability changes. TLC plate preparations and solvent development distances may vary from plate to plate. Spray reagents and the response they produce with different drugs may vary in potency. Therefore, drugs and metabolites may not have exactly the same Rf and spot development on each plate. Relative coefficients of elution and spray reagent responses can easily be compared to standards applied to the plate prior to development.

I-4 **D** **Does the laboratory run appropriate controls or standards to evaluate new lots of spray reagents, solvent systems and/or plates?**

As is recommended for any new batch or lot of reagent in any type of analytical procedure, spray reagents, solvent systems and TLC plates should be evaluated with standards and/or controls before being placed into routine use to ensure they "work" properly.

I-5 **I** **Does the laboratory maintain photographic, photocopy or traced records of thin layer chromatographs?**

A standardized system should be used to record TLC results as they appear on the plate, preferably for both known and unknown detected substances. These records should also include standard and control results. Photographic record keeping procedures should be checked for trueness of color. If the recording system is not photographic, some indication of the colors of the spots should be documented, for example by using a color diagram or colored pencils.

Section J: GAS CHROMATOGRAPHY

J-1 **I** **Does the laboratory have a written procedure for the set-up and operation of the gas chromatograph(s)?**

The SOP should contain sufficient information and directions to enable the operator to set up and adjust each instrument for the required assay. This may include the normal operating conditions for the assay, any adjustments that need to be made (e.g. NP bead current offset, carrier gas flows) or criteria met (e.g. minimum response for a designated analyte). It may also include a description of the settings for any software used for the acquisition and/or processing of analytical data. Settings and descriptions that are critical to or necessary for the individual assays should be described in the method. However, more general descriptions of instrument set-up and operation may be described in a separate section of the SOP. Preventive maintenance that should be performed (e.g. replace septum, cut front of column) may be listed in the individual assays, especially if it is critical to the assay (e.g. replacing the injection port liner prior to each run may be important for some assays). Routine preventive maintenance may often be described in a separate section of the SOP, or in a logbook or chart that stays with the respective instrument.

J-2 **D** **Does the laboratory monitor and record the conditions of the gas chromatograph(s), including the detector response, daily or before each analytical run, if less frequently?**

Where appropriate, operating checks should be made before routine samples are injected. For many instruments and assays this may simply include a "test" injection of one of the calibrators or controls. If it is a frequently run assay, the response can be compared to with that of the previous run, or at least evaluated for appropriate response. Where NP detectors are used, it is appropriate to monitor, and if necessary adjust, the detector offset at least daily or when used.

J-3 **I** **Does the laboratory have written criteria for corrective action for unacceptable instrument performance?**

The SOP should contain some basic guidance on how to troubleshoot problems. In most instances this will be described as part of a section on the set-up and operation of the particular instrument and may be general in nature (e.g. no GC peaks; peaks too small; retention times irreproducible etc). More extensive troubleshooting may be referenced to the appropriate manufacturers manual. However, this should not replace the laboratory's own basic guide which should be in the SOP. Sometimes, corrective action may also be described as part of the individual analytical method, especially where a problem is commonly associated with a particular analyte (e.g. one prone to tailing and adsorption).

J-4 **D** **Does the laboratory evaluate and document the performance of new columns before use?**

Evaluation of new columns may, involve running an appropriate extracted or unextracted standard and comparing the results against a previous run. As a minimum, this may be done immediately prior to running the next assay. Some laboratories may chose to evaluate columns using a cocktail of drugs, some of which are known to be susceptible to adsorption and tailing on columns with active sites. Documentation of column performance may form part of the analytical record, or may be kept as a separate log.

Section K: HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC) and CAPILLARY ELECTROPHORESIS (CE)

K-1 **I** **Does the laboratory have written procedures for the set-up and operation of HPLC and CE instrument(s)?**

The SOP should contain sufficient information and directions to enable the operator to set up and adjust each instrument for the required assay. As a minimum this should include the normal operating conditions for the assay, any adjustments that need to be made (e.g. UV wavelength, mobile phase flow) or criteria met (e.g. minimum signal to noise or peak response for a designated analyte). This should include a description of the settings for any software used for the acquisition and/or processing of analytical data. Settings and descriptions that are necessary for the individual assays should be described in the appropriate method. However, more general descriptions of instrument set-up and operation may be described in a separate section of the SOP. Preventive maintenance that should be performed (e.g. replacing filters, changing guard column) may be listed in the individual assays. Routine preventive maintenance may often be described in a separate section of the SOP, or in a logbook or chart that stays with the respective instrument.

K-2 **D** **Does the laboratory monitor and document the performance of the HPLC and CE instrument(s) at specified intervals?**

The performance of the instruments should be monitored at designated intervals. This may include the evaluation signal-to-noise for known standards, lamp checks and if used may include verification of the accuracy or reproducibility of solvent proportioning ratios. For HPLC daily maintenance may include the routine conditioning of the analytical column by pumping solvent through until the baseline is stable. Some laboratories may replace the first few mm of packing in an HPLC column if performance is deteriorating. Preventive maintenance will normally be recorded in a logbook or separate sheet that is kept with the instrument and filed periodically. Records of some operating checks may be kept with the relevant analytical record (e.g. chromatogram of unextracted or other check standard)

K-3 **I** **Does the laboratory have written criteria for corrective action for unacceptable instrument performance?**

The SOP should contain some guidance on how to troubleshoot problems. In most instances this will be described as part of a section on the set-up and operation of the particular instrument and may be general in nature (e.g. absence of HPLC peaks; poor signal-to-noise; variable retention times, etc). More extensive troubleshooting may be referenced to the appropriate manufacturers manual. However, this should not replace the laboratory's own basic guide which should be in the SOP. Sometimes, corrective action may also be described as part of the individual analytical method, especially where a problem is commonly associated with a particular analyte (e.g. one prone to adsorption and tailing).

K-4 **D** **Does the laboratory evaluate and document the performance of new columns before use?**

Evaluation of new columns may involve running an appropriate extracted or unextracted standard and comparing the results against a previous run. As a minimum, this may be done immediately prior to running the next assay. Some laboratories may chose to evaluate columns using commercial HPLC column performance solutions, some components of which are known to be susceptible to adsorption and tailing on columns with active sites. These checks may form part of the analytical record.

K-5 **D** **If the laboratory recycles the mobile phase, are there procedures for evaluating acceptability?**

For isocratic assays, many laboratories recycle HPLC solvent *in situ* by placing the waste line for the detector into the reservoir for the pumps. If this is done simply while conditioning a column, no special precautions or checks are usually warranted. However, if this is done during routine injections, periodic checks should be made to ensure that the waste analytes, although diluted, do not cause the background to rise to an unacceptable level. For example, one check is to set a limit on the detector zero offset.

Section L: GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC/MS) and LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC/MS)

L-1 **I** **Does the laboratory have written procedures for the set-up and operation of the GC/MS and LC/MS instrument(s)?**

The SOP should contain sufficient information and directions to enable the operator to set up and adjust each instrument for the required assay. As a minimum this should include the normal operating conditions for the gas chromatographic portion of the assay, plus other routine MS tuning and checks which need to be made (e.g. PFTBA tuning, ion source pressure, air and water checks). This should include a description of the settings for any software used for the acquisition and/or processing of analytical data. Settings and descriptions that are critical to or necessary for the individual assays should be described in the method. However, more general descriptions of instrument set-up and operation may be described in a separate section of the SOP. Preventive maintenance that should be performed may be listed in the individual assays, especially if it is critical to the assay. Routine preventive maintenance such as ion source or ion trap cleaning (GC/MS) or spray chamber cleaning (LC/MS) may often be described in a separate section of the SOP, or in a log-book or chart which stays with the respective instrument.

L-2 **I** **Does the laboratory maintain records of mass spectrometric tuning?**

Records of MS tuning records should be maintained. This is so the laboratory can demonstrate that the instrument was operating correctly on the day the assay was run. Hard copies of MS tuning records are typically kept in chronological order in a folder or binder for easy review if a problem subsequently developed. However, an electronic record should also be satisfactory, particularly if the records are in a database format so that they may be searched or graphically displayed. LC/MS instruments are usually more stable than GC/MS instruments and therefore full tuning each day is usually not necessary.

L-3 **E** **Does the laboratory have written criteria for acceptable mass spectrometric tuning?**

The laboratory should have predetermined MS tuning criteria for routine operation. Typically, quadrupole GC/MS instruments are tuned using perfluorotributylamine (PFTBA): the m/z 69 should be 100%, m/z 219 >35% and m/z 502 >1%. However, if the laboratory is performing selected ion monitoring assays, selected tuning may be performed (e.g. to favor the high mass region). Criteria should normally be set to monitor the amount of air and water in the system (ie. as a measure of whether the system is "leaking"). Typically, nitrogen (as m/z 28) is <10% and water (m/z 18) is <5%, relative to m/z 69 of PFTBA. The manufacturer usually identifies LC/MS tuning criteria.

L-4 **I** **If the tuning is unacceptable, is corrective action taken and documented?**

If the tuning parameters are outside the acceptable values set by the laboratory, there should be evidence of corrective action. Sometimes this is indicated directly on the MS tuning records. Often, the corrective action is recorded in a logbook or service record.

L-5 **I** **If the laboratory uses full scan mass spectral identification through library searching, are there written criteria for acceptability?**

This is a difficult area to define, particularly in terms of a mathematical fit or "quality match". However, as a minimum, operators should ensure that all of the diagnostic ions present in the reference spectra are present in the reference spectrum are present in the unknown. Relative abundances of the diagnostic ions, as well as relative retention times must always be considered in designating a "positive" match.

L-6 **E** **If the laboratory uses selected ion monitoring for identification, does it compare ion ratios and retention times between calibrators, controls and unknowns?**

If the laboratory uses selected ion monitoring to simultaneously identify and quantitate certain analytes, whenever possible, three ions should be monitored for the analyte and two ions for the internal standard. For GC/MS, the qualifying ions should be ±20% of the target ion, relative to a calibrator or other standard. Typically, retention times should be ±2% relative to a calibrator or standard included in the same run. Identification and measurement of suitable secondary ions may be more difficult with LC/MS, and ion ratios may be less stable or be more concentration-dependent.

If selected ion monitoring is being carried out for quantitation only, and the analyte has already been identified separately, the above criteria may be considered desirable rather than essential. It is realized that for some methods (e.g. NCI, PCI) it may be difficult to chose multiple ions. Even with EI ionization, some spectra may contain only one or two ions that are >5% relative abundance. Therefore, additional or complimentary methods of identification become important. However, measurement of ion ratios is encouraged where possible. Although LC/MS spectra tend to give simpler spectra than with EI ionization, it is often possible to alter the "fragmenter voltage" or "cone voltage" in order to induce fragmentation, and therefore production of secondary ions.

Similar principles will apply to other chromatographic interfaces (e.g. capillary electrophoresis - CE/MS).

Section M: DATA REVIEW

M-1 **I** **Is there evidence that prior to issuing the final report, the following has been reviewed by a qualified individual, and that the review was documented?**

chain of custody documentation _____
validity of the analytical data _____
quality control _____
final report _____

No matter how good are the abilities of the laboratory to conduct accurate and precise analytical work, those efforts will be defeated unless the appropriate records are carefully reviewed for accuracy and completeness before the final report is issued. The SOP must include a data review procedure. This should include chain-of-custody documentation, all qualitative and quantitative data, including relevant quality control, in addition to a clerical check of the final report. In most instances, the individual who signs the toxicology report will conduct the final review. However, in some circumstances, particularly in larger laboratories, responsibility for part of the review may be delegated to quality assurance or other qualified personnel. Different aspects of the review may be conducted by different people. A "qualified" person is defined as someone with sufficient training and experience to perform the stated review. If the laboratory uses a LIMS for data collection, manipulation and reporting, the system should be designed so that data review by appropriate personnel is required before a report can be released.

M-2 **D** **Where appropriate, is the final report reviewed in the light of the case history?**

Wherever possible, analytical results should be reviewed with reference to whatever case history or other information is available. This can be a valuable quality assurance check. For example, if a fatal concentration of a drug were found in an individual who appeared to be the innocent victim of an industrial accident, further review of the analytical data would be warranted. At the very least, review of the case history should ensure that the appropriate toxicants were tested for. If the laboratory is unable to test for certain drugs which were either requested, or that were available and which could be relevant to the death or incident, it maybe appropriate for this to be stated on the report.

Section N: SAFETY

N-1 **E** **Does the laboratory follow good laboratory safety practices?**

It is essential that the laboratory personnel work in a safe and healthy environment. In the event of an accident, proper equipment should be available to render first aid to the victim and prevent harm to others. Safety must be both the individual and collective responsibilities of all laboratory personnel. The safety manual should, at a minimum, address the following:

- specimen handling, including infectious material and the disposal of biological specimens
- handling and disposal of solvents, reagents, and other chemicals
- handling and disposal of radioactive materials
- handling and disposal of laboratory glassware
- responses to personal injuries
- responses to spillage of biological specimens, chemicals, solvents, reagents or radioactive materials
- evacuation procedures
- regulations governing protective clothing, eating, drinking, or smoking in the laboratory.

N-2 **E** **Does the laboratory have a safety manual which clearly defines all safety policies?**

Laboratory personnel must be familiar with the contents of the safety manual and must have easy access to it.

N-3 **I** **Is this manual available to laboratory staff?**

Each laboratory must be aware of and abide by local, state and federal regulations that may exceed minimum standards established on the basis of the above considerations.

N-4 **D** **Are the laboratory's work areas clean and free of clutter?**

General cleanliness and good housekeeping should be apparent. The laboratory should have proper general ventilation and adequate heating, cooling and humidity control. Adequate and proper lighting should be provided for personnel to carry out assigned tasks.

N-5 **D** **Does the laboratory have adequate room to accommodate all technical work, clerical work, administrative functions and storage of chemical supplies?**

Inadequate space reduces the efficiency of laboratory operations and increases the risk of mishandling or contaminating evidence. Inadequate space also reduces personnel morale and thus adversely affects productivity. The physical design of the laboratory should enhance the flow of work from the time of specimen receipt to final disposal. Interrelationship of functional areas should be laid out in a manner that will facilitate the use of equipment and instruments. Each employee should have enough space to accomplish assigned tasks. Sufficient space should be provided for each instrument to facilitate its use and operation. Personnel should have space available for writing reports and other official communications. An area for general supplies and materials intended for immediate use should be available. An area should be provided for laboratory and clerical supplies that are in excess of short-term use.

SECTION SUMMARIES

For both the Inspector's Checklist and the Self Evaluation Checklist, all sections have a summary page at the end, with the following format:

General Comments:

Recommended Corrective Action:

Suggestions For Improvement (non-mandatory):

In addition, there is a final summary page to allow the inspector to summarize their findings and overall opinion of the laboratory, or in the case of the laboratory director, to summarize corrective action or other changes that may have taken place since the last inspection.