Washington State Patrol

TOXICOLOGY LABORATORY DIVISION

Testing - Quality Assurance Manual

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1 QUALITY MANAGEMENT SYSTEM

1.1 POLICY

The Washington State Patrol (WSP) Toxicology Laboratory Division (TLD) has developed and maintains a quality management system (QMS) appropriate to the testing work performed by the Laboratory. The TLD will document its policies, programs, procedures and instructions to the extent necessary to assure the quality of the test results. The system's documentation will be communicated to,understood by, available to, and implemented by the appropriate personnel. The QMS policies, procedures and objectives are defined in this Quality Manual.

1.1.1 Annual: Annual in this manual refers to the calendar year unless otherwise specified.

1.1.2 Testing: For the purposes of this manual, any and all testing functions performed by the TLD (testing of biological specimens for the presence of alcohol and/or other drugs), unless otherwise specified.

1.1.3 Quality: Adherence to generally recognized standards of good laboratory practice.

1.1.4 Quality Assurance (QA): Those processes and systematic actions necessary to provide confidence that the laboratory's work product and services will satisfy given requirements for quality.

1.1.5 Quality Assurance Manager: The designated individual with oversight of the QA Program for the TLD.

1.1.6 Quality Assurance Program: A planned system of activities describing requirements for forensic analyses and reporting, the purpose of which is to provide confidence that the work product and services provided by the TLD are scientifically sound and valid.

1.1.7 Quality Assurance Records: Records, logs, worksheets and electronic files that provide documented support of conformity to the QMS. These records include, but are not limited to, method and equipment validation documents, equipment verification records, reagent and chemical logs, training records, proficiency and competency test records, courtroom testimony monitoring records and audit records.

1.2 DEFINITIONS

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1.2.8 Quality Control (QC): Internal activities or activities conducted according to established standards used by the TLD to consistently ensure accurate analytical results.

1.2.9 Quality Management System (QMS): The total organizational structure, responsibilities, policies, procedures, and resources for implementing quality management. This includes all activities which contribute to quality, directly or indirectly.

1.2.10 Quality Manual: A collection of the TLD's quality management system policies and objectives for its testing functions, and how these policies and objectives will be implemented.

1.2.11 Technical Procedures/Training Procedures: Scientific methodologies used in forensic analyses. Written procedures will be prepared for routine tests performed by the TLD. The procedures used may be those developed and validated in–house or by an outside laboratory and the foundational training program required for all qualified forensic scientists, prior to assuming forensic analysis.

1.2.12 Forensic Laboratory Services Bureau (FLSB) Director: The Director with ultimate responsibility over the FLSB, comprised of the Toxicology Laboratory Division, the Impaired Driving Section, the Crime Laboratory Division, and the Standards and Accountability Section.

1.2.13 Toxicology Laboratory Division (TLD) Commander: The Commander who oversees the Toxicology Laboratory Division. Also known as the State Toxicologist.

1.2.14 Laboratory Manager: The individual having overall operational responsibility of the testing laboratory

1.2.15 Supervisors: Individuals with overall technical responsibility of personnel performing testing in the laboratory. Also known as the Forensic Scientist Supervisors (FS-5).

1.2.16 Appointing Authority: For the purpose of this manual, the individual with authority to authorize qualified personnel to perform technical procedures, and remove/reinstate personnel or systems from performing testing. For the TLD, the Appointing Authority is the TLD Commander or designee.

1.3 QUALITY POLICY STATEMENT

The management and personnel of the TLD will operate the testing laboratory according to a documented quality management system, the purpose of which is to provide a framework for producing quality service at all levels of the organization. Management is committed to good professional practice and setting a high standard for the quality of its toxicology testing services. Management is committed to continually improving the quality management system by monitoring its effectiveness through, amongst other things: meeting the training needs of personnel, successful proficiency testing, periodic audits and management system reviews, effective corrective and preventive actions and communication with its customers and staff to identify improvement measures.

TLD personnel are required to familiarize themselves with the quality manual and to implement the policies and procedures contained in that manual as well as those contained in technical documents, forms and other instructions when conducting testing in the Laboratory. By doing so, and by contributing to the objective of continual improvement of the management system, personnel will help to achieve the TLD's standard of service as stated below and affirmed by Management signatories.

Laboratory Standard of Service

The TLD will provide professional, conscientious service to its customers by adherence to: consensus standards for laboratory competence, its own quality management system, and to the laws of the State of Washington. High standards of service will be maintained through diligent attention to all details of testing work performed by the Laboratory, and the TLD will strive to set the standard for this work against which similar programs will be judged.

TLD Commander, State Toxicologist

TLD Quality Assurance Manager

TLD Laboratory Manager

1.4 QUALITY ASSURANCE PROGRAM

The TLD QA program includes all technical and supporting procedures and quality records, which TLD management uses to oversee and review the effectiveness of the program. This ensures that the TLD adheres to all Quality Manual policies and procedures.

1.4.1 Division Quality Management

The TLD Commander, Laboratory Manager, QA Manager and Supervisors are responsible for ensuring that the policies and procedures adopted by the TLD are implemented and integrated into the daily operations of the Laboratory. The QA Manager is also responsible for overseeing, monitoring and ensuring compliance to the QMS.

The main duties of the QA Manager include, but are not limited to:

- Responsibility for the overall QA program of the TLD, including all audits and reviews
- Works to maintain and improve the QA program of the TLD
- Maintaining QMS documents and records
- Monitors criteria compliance for respective accrediting bodies
- Evaluation of compliance to the TLD training programs, ensuring uniform quality of education and training
- Ensure uniform methodology implementation and use within the laboratory
- Ensure that procedures and training manuals for the discipline accurately reflect established standards and comply with accreditation requirements
- Review for adherence to procedure and approval of new methodology, technologies and equipment validations
- Evaluate new analytical procedures, equipment or technologies and oversee their validation and assist with implementation
- Administers and coordinates the TLD's proficiency testing program. This includes documentation and response to the Proficiency Review Committee (PRC)
- Organizes and schedules QA meetings
- Oversight and review of root cause analysis and corrective actions for nonconformities and inconsistencies in all calibration work

The supporting duties of other TLD management include, but are not limited to:

- Coordinating the training and development of each Forensic Scientist from basic development to continuing education
- Monitors the development and implementation of the technical and training manuals
- Review of manuscripts for publications
- Review of research projects

1.4.2 All Technical and Support Staff

It is the role of all technical and support staff to follow technical and laboratory supporting procedures, including the documentation required by the QA Program, and to seek to produce the highest quality work in the most efficient manner possible. This commitment helps the TLD meet the needs of the customer and to demonstrate to the citizens of Washington that the TLD are good stewards of the resources given us.

1.4.3 Division Documents

The list below represents the documentation upon which the QMS is built. The TLD Quality Manual has over-riding authority over all operations and technical manuals. The WSP Regulation Manual has over-riding authority over all TLD Manuals.

WSP REGULATION MANUAL

TLD Testing Quality Manual

- TLD Operations Manual
- TLD Standard Operating Procedures and Policies
- TLD Training Manual
- TLD Safety Manual

1.5 QUALITY SYSTEM RECORDS (Access, Filing, Storage, Retention and Disposal)

Quality system records are any logs, worksheets, electronic files or databases that provide documented support of conformity to the QMS. These records include, but are not limited to:

- Method and equipment validation documents
- Instrument and equipment maintenance and verification records
- Reagent and chemical logs
- Training records
- Proficiency test records
- Competency test completion records
- Courtroom testimony monitoring records
- Chemical inventory records
- Audit records

These records are maintained by TLD staff. Filing, storage and retention of these records are as described below.

1.5.1 Records filed, stored and retained by the TLD QA Manager or designee

- Training completion records
- Proficiency test answer sheets
- Method validation approvals

- Corrective actions
- Policy and Procedure manual document review and approval forms
- Audit records and reports
- Laboratory safety inspection reports
- Official electronic controlled documents/forms
- Equipment validation, performance verification and maintenance records
- Testing files and records, and any associated examination or administrative documentation according to retention schedules
- Chemical and reagent logs and worksheets
- Standards inventory records and verification logs
- Equipment Inventory
- Building maintenance and security records and logs (where applicable)
- Visitor logs

1.5.2 Records filed, stored and retained by TLD Management

- Equipment Inventory
- Building maintenance and security records and logs (where applicable)
- Key control records
- Visitor logs

1.5.3 Records Maintained in Bureau-Wide Databases

- Bureau Library Collection
- WSP BTP Discovery Material Website (WebDMS)

All records shall be legible and shall be stored and retained in such a way that they are readily retrievable in facilities that provide a suitable environment to prevent damage, deterioration or loss.

Records stored electronically shall be stored as to prevent unauthorized access or amendment, and will be routinely backed up to prevent loss.

1.5.4 Archive and Retention of Quality System Records

1.5.4.1 Retention and disposal of quality records will follow the WSP Archive Record Retention Schedule or for a period of one accreditation cycle, whichever is longer. A current copy of the Archive Record Retention Schedule may be found on the FLSB Portal.

2 QUALITY ASSURANCE GUIDELINES

These quality assurance guidelines represent those policies to be adhered to when conducting forensic toxicology testing within the TLD. They should serve as a reference point for answering internal and external questions about quality assurance processes at work in the Laboratory. Unless otherwise defined in a specific procedure, these are the guidelines to be followed for each respective topic.

The guidelines are by no means comprehensive and do not cover all possible quality assurance topics. Forensic Scientists are directed to seek guidance from Supervisors and Management for specific topics not addressed in this or other related documents.

Deviations from these guidelines must be approved by the State Toxicologist, Quality Assurance (QA) Manager, Lab Manager or Supervisor, with the approval recorded in the batch file, case file or other location, where appropriate.

2.1 CONFIRMATION OF POSITIVE RESULTS

2.1.1 The initial identification/detection of drugs or toxins will be confirmed by a second technique based upon a different chemical principle to the initial technique, wherever possible.

2.1.1.1 In circumstances where a confirmatory test cannot rely on a separate chemical principle, chemical modification of the target analyte and analysis by the same technique may be employed. An example would be initial detection by GC/MS and confirmation by GC/MS following silylation or alkylation of the analyte. In such instances it is not acceptable to derivatize the extract used for initial detection unless there is limited sample available.

2.1.1.2 Generally, it is not acceptable to confirm a chromatographic result using the same system with a modification of the separation column polarity/selectivity. In instances where the selectivity difference results in changes in elution order, such as for the dual column analysis of ethanol and other volatiles, then confirmation by this method is appropriate.

2.1.1.3 Confirmation of an immunoassay by a second immunoassay, whether differing in cross-reactivity or not, is not acceptable. Presumptive positives may be reported, provided that wording appears on the final report indicating that the results are not confirmed.

2.1.2 The confirmatory test will, wherever possible, be more selective and sensitive for the targeted analyte than the first test.

2.1.3 Where applicable, mass spectrometry is the preferred method of detection for a confirmatory test.

2.1.4 Confirmation of a positive result will be performed following a fresh sampling of the original specimen.

2.1.4.1 If quantity is limited for the original specimen, a second specimen type or source may be used for confirmation.

2.1.4.2 The re-analysis of the initial extract, even by a method with a different chemical principle, is not acceptable for confirmation.

2.1.4.3 The analysis of a single extract on a dual-detector system is not an acceptable form of confirmation unless the specimen has had a fresh sampling.

2.1.5 When a confirmatory test based on a different chemical principle is not available, it may be acceptable to test a fresh sampling of the specimen, or an alternate specimen, for confirmation by the same method. This is applicable to the most selective of methods such as mass spectrometry and should preferably be supported by case history accompanying the evidence. It is not acceptable for immunoassay.

2.2 BATCH ANALYSIS

2.2.1 Batch analysis of specimens will contain quality controls to monitor method performance.

2.2.1.1 At a minimum the batch must include a negative control and a positive control.

2.2.2 Controls should be matrix-matched to the specimens being analyzed, wherever possible.

2.2.2.1 If more than one matrix is analyzed within a batch, the number and makeup of positive and negative controls will be replicated for each matrix, where practicable.

2.2.3 The number of controls will be dictated by the batch size and the testing type; qualitative or quantitative.

2.2.3.1 Quantitative batch analysis will have at least 10% of the batch (number of case specimen samples) consist of controls.

2.2.4 Quality controls for quantitative batch analysis will bracket the tested specimens to monitor method performance through completion of batch testing.

2.2.4.1 At least one calibrator or positive quality control sample will be analyzed at the end of the batch to verify quantitative method performance.

2.2.5 Analysis of blanks within a batch.

2.2.5.1 Analysis of blanks may be included in the batch, when warranted. Instances that warrant blank injections include the following.

a. After analysis of specimens where high drug concentrations are expected.

b. After analysis of decomposed or otherwise degraded specimens.

c. After analysis of calibrators or controls with high drug concentrations in order to evaluate carryover.

2.2.5.2 Blank injections count toward the total number of analyses or injections when any such number is specified in a policy or procedure.

2.2.5.3 Blank injections may be either blank matrices, extracts of blank matrices, or solvent blanks, whichever is best suited to the analysis.

a. When solvent blanks are included, the solvent will be matched to the solvent used for specimen extracts, unless otherwise specified within a specific standard operating procedure.

2.2.5.4 When a blank is analyzed to evaluate carryover into a case sample, a copy of the blank report will be filed with the result for the case immediately following the blank injection.

2.2.6 Batch size will be limited by any restrictions, either physical or operational, of each method. If a method specifies a limit on batch size then that limit shall not be exceeded.

2.2.7 A record will be produced for each batch analysis indicating the identification and order of analysis for each member of the batch.

2.2.8 All members of a batch will be represented by a report of analysis (however named) which will be provided for any subsequent review for the purposes of evaluating the batch as a whole.

2.2.8.1 The data for each member of a batch, including any blanks, will be retained in the record.

2.3 QUALITY CONTROL SAMPLES

2.3.1 Quality control (QC) samples will be processed in the same way as case specimens during testing.

2.3.2 Each test, whether of a single specimen or of multiple specimens, will contain at a minimum a single negative QC sample and single positive QC sample.

2.3.3 QC samples will be matrix- matched to the specimens being analyzed, wherever possible.

2.3.4 Negative quality control samples

2.3.4.1 Negative QC samples will be prepared in matrices that have been shown through testing to be negative for the drug or toxin under investigation.

2.3.4.2 For qualitative methods, any negative QC sample should give results that show the target compound(s) is absent or below the cutoff threshold.

2.3.4.3 For quantitative methods, any negative QC sample should give results that show the target compound(s) is absent or below the limit of detection for a method.

a. Any criteria for acceptance of a positive result specified in a method, such as ion ratio or retention time tolerance, minimum peak area/height, chromatographic resolution or appearance, must be considered before deeming a negative QC sample unacceptable.

2.3.4.4 Negative QC samples will contain one or more internal standards for quantitative methods. A negative sample without internal standard(s) is defined as a blank.

2.3.4.5 If a negative QC sample indicates the presence of a drug, it will first be assessed for carryover or contamination. This can be done through reanalysis/reinjection of the negative QC. If carryover or contamination is ruled out, then all specimens which identify the presence of that particular drug will be retested. Those specimens which do not identify the presence of that particular drug do not need to be retested for that drug.

2.3.5 Positive quality control samples

2.3.5.1 Qualitative methods

a. Positive QC samples will indicate the presence of the drug or drug class of interest.

b. If the positive QC sample(s) do not indicate drug presence then all specimens will be retested.

2.3.5.2 Quantitative methods

a. Positive QC samples will have concentrations between the lowest and highest calibrator of the method.

b. If a single positive QC sample is used, its concentration will be a value near the midrange of the calibration curve, unless otherwise described in the individual test procedure.

c. If multiple positive QC samples are used, their concentrations will be selected to monitor method performance across the reportable concentration range of the method (e.g. low, mid or high).

d. Positive QC samples will be deemed acceptable if their values lay within ± 20% of their targeted concentration.

e. Criteria for QC samples are described in the individual test procedures

2.3.5.3 Reference material (RM) used to produce positive QC samples

a. Reference material used to produce positive QC samples will be from a different source than that used to produce calibrators. Barring that, the following hierarchy will be used.

b. The hierarchy in preference for positive QC sample reference material is as follows.

- Different supplier from calibrator RM
- Different lot number or production run from calibrator RM
- Different preparation, weighing, dilution of calibrator RM

a. Positive QC samples should not be made from the exact same RM weighing or dilution as that used to produce calibrators.

2.4 CALIBRATORS

2.4.1 The lowest calibrator in a quantitative method will be the lower limit of quantitation (LLOQ) and the highest calibrator will be the upper limit of quantitation (ULOQ).

2.4.1.1 The LLOQ can also be referred to as the limit of quantitation (LOQ).

2.4.1.2 The ULOQ can also be referred to as the limit of linearity (LOL).

2.4.2 The LLOQ or ULOQ may be determined experimentally or administratively. Regardless of how they are determined, the reportable range of values will lie within the limits of the LLOQ and ULOQ.

2.4.3 The calibrators should bracket the anticipated concentrations of target analytes in case specimens as often as possible.

2.4.3.1 If the value of a case specimen is above the highest calibrator then the case specimen should be reanalyzed as a dilution.

2.4.3.2 If the value of a case specimen is below the LLOQ, then a larger volume of the specimen may be extracted and analyzed. Alternatively, a calibrator with a value less than both the LLOQ and the expected specimen concentration can be analyzed. In such an instance, this lowest calibrator must meet all the criteria of an LLOQ and any other criteria for identification specified in the method. Either instance must be approved by Supervisor or TLD Management, and documented in the batch file.

2.4.4 There will be a minimum of three calibrators used to generate calibration curves.

2.4.5 Calibrators will be back calculated against the contemporary calibration curve. The LLOQ is acceptable if its value is within ± 25% of the target. All other calibrators are acceptable if their values are within ± 20% of their targets.

2.4.6 Calibrators for ethanol analysis will be deemed acceptable if their values are within ± 10% of their targets.

2.5 CALIBRATION CURVE OR STANDARD CURVE

2.5.1 Single point calibrations are not encouraged. If single point calibration is necessitated, there must be positive QC samples tested at the extremes of the reporting ranges.

2.5.2 Multi-point calibration curves will be produced that graph area, or height, of analyte to internal standard ratio against calibrator concentration. Calibration curves must contain a minimum of three calibration points.

2.5.2.1 A linear relationship will be the default approach to generating calibration curves.

2.5.2.2 In instances where quadratic relationships are necessitated, they will be verified by the use of sufficient positive QC samples.

2.5.3 Calibration curves may be generated by instrument software or by graphing software.

2.5.4 The correlation coefficient (r^2) of the calibration curve will be used as the criteria for acceptability. The acceptable r^2 value is 0.99 or higher for most methods. There may be methods for which r^2 of 0.98 or higher is minimally acceptable.

2.5.5 Weighting of calibration curve data points will be either equal or inverse of concentration (1/a).

2.5.6 Calibration curves will ignore the origin. The exception to this being blood ethanol/volatiles testing by headspace gas chromatography.

2.5.7 Calibration by standard addition may be employed if necessitated by uniqueness of the specimen although it should be closely monitored for matrix effects by the use of an internal standard.

2.5.8 Excluding calibrators

2.5.8.1 Exclusion of calibration points from the calibration curve is not encouraged and should be an infrequent occurrence. It is preferable to re-analyze a batch wherever possible rather than exclude calibrators.

2.5.8.2 No more than one calibrator may be excluded from a calibration curve. Calibrators are typically considered for exclusion due to one of the following reasons.

a. Low extraction recovery or sample lost during extraction (broken tube, etc.)

b. Incomplete or inefficient injection

c. Calibrator concentration value outside of acceptable range

2.5.8.3 Before excluding a calibrator, it is acceptable to re-analyze/re-inject it to rectify any issue. Any re-analysis should be documented.

2.5.8.4 If the LLOQ is to be excluded, the next highest calibrator will be the lower reporting limit for the test. Any positive samples between the LLOQ and the new lower reporting limit should be re-tested.

2.5.8.5 If the ULOQ is to be excluded, the next lowest calibrator will be the upper reporting limit for the test. Any positive samples between the ULOQ and the new upper reporting limit should be re-tested.

2.5.8.6 There must be a minimum of one positive QC sample within the reporting range of the calibration curve following any exclusion of calibration points.

2.6 DILUTION OF SPECIMENS

2.6.1 When a high concentration of a sample is expected due to case history or the results of an initial test or when a quantitative test result is above the ULOQ for the method, dilution of the sample for confirmatory quantitative testing is justified.

2.6.2 Dilution may be done using negative matrix, deionized water or an appropriate buffer to make up the volume/balance. The selection of a diluent will be made as appropriate to the circumstances or method employed.

2.6.3 Dilution may be done in one of the following ways.

2.6.3.1 Sampling of a fraction of the standard volume followed by volume balance comprised of diluent. (Example: 0.1 mL of blood is sampled, 0.9 mL of certified blank blood is added and the diluted sample is tested.)

2.6.3.2 Sampling of the standard volume, dilution to a selected volume and re-sampling of the dilution at the standard volume. (Example: 1 mL of blood is added to 9 mL of deionized water and 1 mL of the dilution is sampled for testing.)

2.6.4 The dilution of a specimen will be documented and should appear on the resulting report for the test.

2.6.5 Dilution naming will observe the following convention.

2.6.5.1 Dilution of a specimen will be named as a ratio wherein the first number represents the volume of specimen proportional to the second number which is representative of the standard volume. The standard volume of specimen for the test is divided by the second number to obtain the volume of specimen used in the dilution.

a. Example: A 1:10 dilution is used for a test which has a standard volume of 1 mL. 1 mL divided by 10 produces a 0.1 mL volume of specimen used to prepare the dilution. Alternatively if the standard volume is 2 mL, 2 divided by 10 indicates a 0.2 mL specimen volume.

b. Example: A 1:2 dilution of a 1 mL standard volume means that the specimen volume is 0.5 mL and the diluent is 0.5 mL. By this naming convention there is no such thing as a 1:1 dilution.

2.6.5.2 Calculation of a final concentration for a dilution is done by multiplying the second number of the ratio by the resulting concentration of the dilution.

2.6.6 A dilution result is considered acceptable if the uncorrected concentration lies within the LLOQ and ULOQ.

2.6.7 When multiple dilutions are used, the result of the least diluted specimen lying between the LLOQ and ULOQ will be reported. Generally, there should be good agreement between the corrected concentrations for multiple dilutions of a single specimen.

2.6.8 When multiple analytes with varied concentrations are being evaluated in a single test, it is recommended that an undiluted, standard volume be tested alongside any dilutions. This is done to establish that any negative test result is due to the absence of the analyte in the specimen rather than its presence being diluted below a reportable concentration. Such testing should be done with consideration of carryover from the undiluted specimen. This is typically observed when both an analyte and a metabolite of the analyte are tested.

2.6.9 It is recommended that diluted specimens be tested toward the end of a batch analysis and that if multiple dilutions of a single specimen are included, that they be tested in order of most to least dilute.

2.7 INTERNAL STANDARD

2.7.1 The use of internal standard quantitation is preferred.

2.7.2 The internal standard should be selected based upon chemical and physical property similarity to the analyte(s) being tested.

2.7.3 If the analyte(s) being tested is to be derivatized, an internal standard should be selected which will undergo similar derivatization. Preferably the same degree of derivatization and the functional group(s) derivatized will be the same between analyte and internal standard.

2.7.4 Stable isotope standards, most commonly deuterium-labeled, are the preferred internal standards for quantitative testing. A non-labeled compound is an acceptable alternative as internal standard.

2.7.5 Deuterated internal standards

2.7.5.1 Only high purity deuterated internal standards should be selected.

2.7.5.2 A deuterated internal standard should not produce a response which would be interpreted as a positive result for the non-labeled analyte in a negative specimen, nor should it interfere with the signal(s) monitored for the non-labeled analyte.

2.7.5.3 Generally, the higher the amount of deuterium substitution the better the internal standard. In practice, this may be limited by factors of availability and cost.

2.7.6 There is no limit on the number of internal standards that may be employed for a quantitative test.

2.7.7 The internal standard is to be added to all calibrators, negative and positive QC samples and specimens being tested.

2.7.8 The internal standard is to be added as early as possible in the sample preparation scheme and always prior to buffering and extraction. The addition of internal standard after initial extraction is not approved.

2.7.9 The internal standard may be used to assess the recovery of an extraction or the efficiency of automated instrumental analysis. Generally speaking, if a specimen's internal standard response, measured by peak area or height, is less than 50% of that measured for calibrators and QC samples then follow up may be warranted. Follow up may include any of the following steps.

2.7.9.1 The extracted specimen may be reinjected. This should be done as soon as possible relative to the original injection. Any reinjection of the specimen should be documented.

2.7.9.2 A fresh sampling of the specimen is retested.

2.8 RE-INJECTION

2.8.1 There may be circumstances in which reinjection of either selected members or the complete testing batch is necessary. When this occurs, the reinjection will be documented in such a way as to unambiguously identify any original result from a reinjected result.

2.8.2 Reinjection will be performed sparingly and with recognition of the impartiality that must accompany the forensic testing process.

2.8.3 Situations that may arise for which reinjection would be considered include:

2.8.3.1 The retention time of an analyte or internal standard is outside of specification.

2.8.3.2 Peak resolution or shape is affected by other closely eluting peaks.

2.8.3.3 A mass spectrum for an analyte contains interference, possibly in the absence of chromatographic interference.

2.8.3.4 Analyte or internal standard abundance is lower than expected.

2.8.3.5 An autosampler fault causes an injection to fail or be sub-optimal. (e.g. needle misalignment or damage)

2.8.3.6 Extract evaporation prior to injection.

2.8.3.7 Carryover is identified.

2.8.3.8 Instrumental conditions (examples: power loss, vacuum loss, loss of network connection, carrier gas depletion, mobile phase leak, etc.)

2.8.4 The reason for the reinjection(s) will be recorded in such a way as to clearly explain the cause, the specimens, calibrators or QC samples affected and the results of the reinjection(s).

2.9 CARRYOVER

2.9.1 Carryover is manifested in one of three ways. An analyte appears in the test result of a specimen which is inherently negative. The concentration of analyte in a specimen is artificially elevated in the test result. Interferences, not native to the specimen, confound normal specimen testing (i.e. decomposition byproduct carryover).

2.9.2 Carryover may occur by one or a combination of the following mechanisms.

2.9.2.1 Automated sampling systems retain the analyte from another source and contaminate the specimen's test result.

a. This can arise from incomplete cleaning or purging of the automated equipment (syringe, sampling loop or sampling probe) between tests or from the analysis of high analyte concentration specimens prior to the analysis of the affected specimen.

2.9.2.2 Analyte is retained in the testing system separate from the sampling unit. This is typically tied to incomplete elution of the analyte in chromatographic systems and can be identified by retention time variance. It can also originate from the sample introduction system and associated pneumatics such as found in a split/splitless injector for a GC. In these situations, there may not be any observable variance in analyte retention time.

2.9.2.3 Previously tested specimens contain interferences that are not the targeted analyte but that are retained in the testing system and interfere with normal testing of a subsequent specimen. This is typically seen when decomposed or otherwise degraded specimens are analyzed.

2.9.3 Eliminating carryover prior to testing

2.9.3.1 Dilute specimens that are known or expected to contain high concentrations.

2.9.3.2 Select appropriate wash solvents and wash durations for sampling systems.

a. Solvents should be selected which are compatible with the sampling and testing systems and which are capable of properly solubilizing both targeted analytes and any potential decomposition byproducts.

b. The duration or extent of any wash cycles should be sufficient to effectively purge the sampling system of carryover.

c. For batch analyses, sufficient volumes of wash solvents should be provided to allow for adequate sampling system washing throughout the length of the batch.

2.9.3.3 Ensure effective sample preparation.

2.9.3.4 Testing decomposed specimens with sufficient blanks and near the end of any batch analysis list.

2.9.4 Assessment of carryover during testing can be done through the testing of blanks between specimens, calibrators or QC samples.

2.9.4.1 Solvent blanks should be selected with consideration of their compatibility with the testing system and their ability to mimic a typical injection. Due consideration of a solvent's polarity and ability to solubilize the analyte or decomposition byproduct should be made.

2.9.4.2 The presence of a targeted analyte in a solvent blank is not evidence that a subsequent specimen was subjected to carryover. The intensity of analyte in the blank may warrant that conclusion but it may also indicate carryover to a minimal degree that has no effect on a subsequent sample's result. Each situation must be carefully evaluated and the method validation data can be consulted to determine the likelihood of significant carryover. It is prudent to conclude that there is carryover of analyte in a positive specimen when it is preceded by an analyte-positive blank. In situations with multiple targeted analytes, only those present in the preceding blank are necessarily of concern and warrant follow-up.

2.9.5 Reconciling carryover

2.9.5.1 If carryover is suspected in a specimen result, it is appropriate to reanalyze/reinject that specimen in the absence of the potential source of the carryover. Typically, a blank injection is made using either a blank which did not show evidence of carryover or a fresh solvent blank. This is followed by reinjection of the specimen. This approach may not be appropriate where the carryover was of a very large scale. In such an instance it is more appropriate to make a fresh sampling of the evidence and retest it in the absence of the source of carryover.

2.9.5.2 If reanalysis/reinjection does not sufficiently rule out carryover, then a fresh sampling of the specimen and retesting should be conducted. Any re-sampling and retesting should be conducted in such a way as to prevent the original suspected carryover from reoccurring. This is accomplished through the use of sufficient blanks, by changing the order of analysis or by the use of diluted specimens where appropriate.

2.9.5.3 All reconciliation of carryover should be documented and available for any subsequent reviews.

2.10 CHROMATOGRAPHY

2.10.1 The majority of the confirmatory methods in the laboratory are based upon some type of chromatographic system. Chromatography is a powerful separation technique, not a detection technique. As a separation technique, chromatography may produce data rich with supporting information for a particular specimen. Additional metabolites, pharmaceutical excipients or co-administered drugs may be identified through a careful examination of the other chromatographic peaks present in a targeted analysis. However, as a separation technique, chromatography is also capable of resolving peaks of interest from endogenous or otherwise extraneous peaks of little or no informational value. The mere existence of a peak in a chromatogram of a biological specimen does not imply that it is either relevant or worthy of additional attention.

2.10.2 Chromatography is most useful when it provides clearly defined, well resolved and reproducible data. Evaluation of chromatographic data is based upon several factors, retention time precision, peak resolution and peak symmetry.

2.10.3 Chromatographic retention time

2.10.3.1 Retention time for a chromatographic peak is measured at the peak apex. This is normally done through automated peak integration software contained with the instrumental operating system.

2.10.3.2 Retention time acceptability for a peak in a specimen is based upon reproducibility against a reference, typically that of the same peak appearing in a calibrator or control.

a. A retention time for a specimen peak is generally acceptable if it is within ± 2% of the time noted for the reference retention time. For high performance liquid chromatography, the tolerance may be as high as ± 5%.

b. The reference retention time, unless stated otherwise in a particular method, will be derived from the calibrator that is closest to one order of magnitude above the LLOQ. For example a calibration curve consisting of 0.01, 0.05, 0.075, 0.25 and 0.75 mg/L will have the retention time reference taken from the 0.075 mg/L calibrator.

c. Reference retention times may be averaged from all calibrators, only if specified in a particular method.

2.10.4 Peak resolution

2.10.4.1 Chromatographic peaks of interest should be well-resolved from other peaks in the chromatogram. A distinction should be drawn between the raw chromatographic data and any extracted data used to create additional chromatograms. While a chromatogram generated from all collected data may indicate poor peak resolution, filtering the relevant data can reveal a well-resolved acceptable peak of interest. For example, collection of full scan mass spectral data of MDMA and its internal standard MDMA-D_5 may produce two poorly resolved peaks; extraction of those ions specific to either compound will produce two distinct and well resolved peaks free from interference.

2.10.4.2 For two closely eluting peaks, the peak of interest is considered resolved if the valley between the two peaks is ≤ 10% of the peak height of interest.

2.10.5 Peak symmetry

2.10.5.1 A chromatographic peak of interest should be symmetrical in appearance (also referred to as bell-shaped).

2.10.5.2 Peak tailing or fronting should be minimized as much as possible as either condition can make quantitative accuracy or low-level measurement difficult. With some analytes and chromatographic systems, peak tailing may be unavoidable and may not necessarily complicate accuracy. In these situations, the results of positive QC samples will provide a reference for acceptability of quantitative results.

2.10.5.3 Principles of chromatographic acceptability should be applied to internal standard peaks as well as targeted analyte peaks. Often the examination of internal standard peaks can provide information on the entire chromatographic system as the internal standard provides a consistent gauge of instrumental performance from specimen to specimen or injection to injection.

2.11 MASS SPECTROMETRY

2.11.1 Mass spectrometry (MS) is a detection method that is usually employed in a toxicology laboratory as a hyphenated separation instrument/detector combination. Mass spectrometers may be coupled to gas chromatographs (GC) and operated in either electron ionization mode (EI) or chemical ionization mode (CI) with choices of reagent gas supply. Mass spectrometers employed for GC/CI-MS or coupled to high performance liquid chromatographs (LC/MS) generally produce softer, lower energy ionization and fewer mass-to-charge fragments compared to GC/EI-MS.

2.11.2 For purposes of screening, mass spectrometry is usually operated in full scan mode. For quantitative, confirmatory methods, selected-ion-monitoring (SIM) is most commonly used.

2.11.3 Qualitative identification

2.11.3.1 The analyte in the specimen should have its mass spectrum compared to the mass spectrum of a reference material, a spectrum from a reference library, or both. When using a library match, spectrum agreement should be ≥75 wherever possible, taking into consideration the presence and abundance of major and diagnostic ions specific to that compound (an extracted ion match may be necessary). A reference spectrum for the compound found in a published article, research paper or other reference material may be acceptable if an electronic library match is not feasible, provided the source is documented.

2.11.3.2 Wherever possible, the identification should also account for retention time matches to that of a standard or positive control.

2.11.4 Quantitative identification

2.11.4.1 When SIM is employed, a minimum of three ions must be monitored for each analyte and two for each internal standard. In certain circumstances there may not be additional diagnostic ions available for SIM monitoring (examples: GC/CI-MS or LC/electrospray ionization MS). This may be acceptable, provided that compound identification has been accomplished by other means, and if the ions monitored by SIM are unique to the analyte.

2.11.4.2 The relative abundances of SIM ions are to be monitored and compared to those found in the reference material. This is commonly referred to as measuring ion ratios.

2.11.4.3 The reference ion ratios, unless stated otherwise in a particular method, will be derived from the calibrator that is closest to one order of magnitude above the LLOQ; similar to the designation used for retention time references.

2.11.4.4 For both analytes and internal standards, a quantification ion will be selected for all calculations of analyte concentration.

2.11.4.5 All other selected ions are termed qualifier ions and their abundances relative to the quantification ion is what is used to produce ion ratios.

2.11.4.6 Ion ratio acceptability is defined as ± 20% relative to the reference ratio for a given ion pair. Ion ratios for LC/MS data may be more concentration-dependent than for GC/MS data, therefore ± 25% may be specified in a particular LC/MS method.

2.12 MANUAL INTEGRATION

2.12.1 Integration of chromatographic peaks is performed by the data analysis software accompanying each instrument. Peak integration is automated and the settings for integration are user-defined and applied in the same way to all data collected for a particular batch. Occasionally, the automated process may need to be supplemented by manual integration of select chromatographic peaks. Manual integration should be infrequent; however each chromatogram is unique and as such presents the automated

integration function with a unique collection of peak slopes, inflection points and baseline. Manual integration may be used to properly define the peak when it is clear that automated integration is either improperly including or excluding data in its definition of the peak.

2.12.2 When manual integration is employed it should be indicated in the record so that subsequent data review can be informed of its use. This can be accomplished by inclusion of original and manually integrated chromatograms or through flagging of data as having been manually integrated.

2.13 TRUNCATING AND SIGNIFICANT FIGURES

2.13.1 Quantitative results will be truncated, never rounded, to the final number of reported significant figures.

2.13.2 Quantitative results will be reported to no more than two significant figures. If it can be demonstrated that a particular measurement has accuracy greater than two significant figures, as specified in the applicable test procedure, then it may be reported as such.

2.13.3 If duplicate results are measured for a specimen, they should agree to within ± 20% of their mean, unless specified otherwise in a procedure. The quantitative result will be the lesser of the two values truncated to two significant figures. Note: Duplicate results may be two individual samplings from the same specimen within the same batch or from two different batches (one serving as identification and one as confirmation/quantification).

2.14 REPORTING

2.14.1 The specimen(s) tested, the method of testing, and the results will be reported accurately, clearly, unambiguously and objectively.

2.14.2 All positive results for urine samples, whether qualitative or quantitative, will be reported as "Positive". In instances where a urine concentration may be used to differentiate between an endogenous or exogenous source of an analyte, a value may be reported.

2.14.3 Qualitative

2.14.3.1 Negative qualitative results will be reported as "None Detected". If clarification is warranted or requested by a customer, the result may be reported as "None Detected at x", where x is the lower reporting limit for the particular test.

2.14.3.2 Positive qualitative results will be reported as "Positive".

2.14.4 Quantitative

2.14.4.1 Negative quantitative results will be reported as "None Detected". If clarification is warranted or requested by a customer, the result may be reported as "None Detected at x", where x is the lower limit of quantitation for the particular test.

2.14.4.2 Positive quantitative results will be reported to no more than two significant figures in units of mg/L for fluids (or ng/mL for analytes with a LLOQ, 0.10 mg/L) and mg/kg for tissues, unless otherwise indicated in the individual test procedure.

2.14.4.3 When there is a valid result for an analyte from more than one test batch, the value from the first test performed is reported.

2.14.4.4 If two samplings from the same specimen are analyzed within the same test batch, the two results must agree within ±20% of their mean, with the lesser of the two values is reported.

3 EQUIPMENT MAINTENANCE

This chapter describes the calibration, maintenance, traceability and records that are maintained for equipment used by the TLD. Forensic Scientists are directed to seek guidance from Supervisors and management for specific topics not addressed in this or other related documents.

Deviations from these guidelines must be approved by the State Toxicologist, Laboratory Manager or the QA Manager and the approval recorded in the maintenance/equipment log or other location where appropriate.

3.1 DEFINITIONS

3.1.1 Equipment: Equipment refers to those devices used during the course of testing that support the test result or measurement and that may or may not affect the outcome. In general, equipment is distinct from the instrumentation used to conduct a test on evidence.

3.1.2 Maintenance: Those activities associated with the proper handling and operation of equipment that help to ensure its continued suitability for use. Maintenance may include a repair or, as more frequently encountered, an ongoing process of preventative care done at a defined interval.

3.1.3 Calibration: Operation that, under specified conditions, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication (VIM 2008).

3.2 CALIBRATION OF EQUIPMENT

3.2.1 External calibration of equipment will only be conducted by an approved calibration supplier.

3.2.2 Approved calibration suppliers will have been evaluated for the competence and traceability of their calibrations.

3.2.3 Accreditation to ISO/IEC 17025:2005 is considered evidence of the competency and traceability of a calibration supplier's services; however, the scope of the calibration services must be appropriate to the equipment undergoing calibration.

3.2.4 Up-to-date certificates and scopes of accreditation will be maintained for all approved calibration suppliers.

3.2.5 Internal calibration of equipment will be conducted according to written procedures. When internal calibration fails to meet a standard of acceptability, the equipment shall be transferred to an external calibration supplier.

3.3 BALANCES

3.3.1 Calibration will be performed on an annual basis for any analytical or top-loading balances. Calibration will be performed by an approved calibration service provider.

3.3.2 Maintenance of balances will include the following:

3.3.2.1 Keep the weighing stage clean and dry

3.3.2.2 Isolate the balance from vibration and air currents

3.3.2.3 Ensure the balance is level

3.3.3 Intermediate checks on balance accuracy will be made quarterly by the QA Manager or designee.

3.3.3.1 Analytical balances will be checked using ASTM Class-1 mass standards. Mass tolerances will be 100 (±0.01) mg, 10 (±0.01) mg and 5 (±0.01) mg.

3.3.3.2 Top-loading balances will be checked using NIST Class F mass standards. Mass tolerances will be 100 (±0.002) g, 10 (±0.002) g and 5 (±0.002) g.

3.3.3.3 If tolerances are not met for an intermediate check, internal re-calibration functions for the balance may be employed or an approved calibration service provider may perform re-calibration.

3.3.4 Records of annual calibration, intermediate checks and of repairs will be retained in the equipment log.

3.3.5 Mass reference standards will be certified every 3 years by an approved service provider.

3.4 CENTRIFUGES

3.4.1 Calibration of laboratory centrifuges is not required. There are no critical speeds associated with any procedure and as such all references to centrifugal force, force of gravity or revolutions per minute (rpm) that appear in laboratory procedures are merely offered as recommendations. Additionally, any timeframes for the use of centrifuges in a particular procedure may be lengthened or shortened as needed to obtain the results sought (separation of liquid layers, concentration of solids dispersed in liquids, etc.)

3.4.2 Maintenance of centrifuges will include the following.

3.4.2.1 Keeping the centrifuge clean and dry.

3.4.2.2 Using the centrifuge with the rotor properly balanced

3.4.2.3 Operating the centrifuge on a level surface

3.4.3 Intermediate checks on centrifuge performance are not required.

3.4.4 Any records of centrifuge repair will be maintained in the equipment log.

3.5 DILUTERS

3.5.1 Diluters (a.k.a. diluter-dispensers) will be calibrated on an annual basis. Calibration may be performed by laboratory staff or by an approved calibration service provider.

3.5.2 Maintenance of diluters will include the following.

3.5.2.1 Examination of tubing for kinks and replacement when noted.

3.5.2.2 Examination for microbial growth and cleaning/disinfection, if noted.

3.5.2.3 Checking for fluid leaks.

3.5.3 Intermediate checks on diluter performance are not required.

3.5.4 Records of diluter certification and repairs will be maintained in the diluter calibration log.

3.6 EVAPORATORS

3.6.1 Calibration of evaporator temperatures is not required unless intermediate checks or normal operational use identify complete failure of the heating system requiring repair. Any temperatures associated with technical procedures that use an evaporator are not critical temperatures.

3.6.2 Maintenance of evaporators will include the following

3.6.2.1 Maintaining proper volume of water in the heated space of the evaporator.

3.6.2.2 Periodic replacement of water and use of anti-algae additives in the water.

3.6.2.3 Keeping the outside of the evaporator clean.

3.6.3 Intermediate checks of evaporator temperature will be conducted annually by the QA Manager or designee.

3.6.3.1 The temperature of the evaporator heated space will be measured using a NIST traceable thermometer.

3.6.3.2 The evaporator water will be heated to 40° C and the temperature verified to be within ± 4° C.

3.6.3.3 If the temperature is outside of tolerance, the evaporator will be taken out of service pending repair.

3.6.4 Records of evaporator maintenance, intermediate temperature checks and repairs will be maintained in the equipment log.

3.7 HEATING BLOCKS, SAND BATHS, INCUBATORS, OVENS

3.7.1 Calibration of heating sources is not required. There are no critical temperatures associated with any procedure and as such all references to temperatures employed for periods of incubation appearing in laboratory procedures are merely offered as recommendations. Heating sources may have integrated thermometers or supplemental thermometers.

3.7.2 Maintenance of heating sources will include the following

3.7.2.1 Equipment will be kept clean.

3.7.2.2 Equipment faults will be identified and the equipment removed from service until repair.

3.7.2.3 Any supplemental thermometers used to estimate temperature will be maintained in good working order and replaced if damaged or found to be outside of tolerance by intermediate checks.

3.7.3 Intermediate checks of heating source temperature and supplemental thermometers will be conducted annually by the QA Manager or designee.

3.7.3.1 The temperature of the heating source will be measured using a NIST traceable thermometer.

3.7.3.2 The heating source will be set to the desired temperature and it, or supplemental thermometer, will be measured to within ± 4°C.

3.7.3.3 If the temperature is outside of tolerance, the heating source will be taken out of service pending repair. If measured using a non-integral thermometer, the thermometer will be replaced.

3.7.4 Records of intermediate temperature checks, repairs and supplemental thermometer replacement will be maintained in the equipment log.

3.8 HYDROGEN GENERATORS

3.8.1 Maintenance of hydrogen generators will include the following as needed.

3.8.1.1 Add deionized water.

3.8.1.2 Change moisture filter.

3.8.1.3 Replace deionizer bag/cartridge according to manufacturer recommendations.

a. Records of hydrogen generator maintenance and repairs will be maintained in the equipment log.

3.9 NITROGEN GENERATORS

3.9.1 Maintenance of nitrogen generators will include the following.

3.9.1.1 Replacement of pre-filters annually.

3.9.1.2 Replacement of any additional downstream filters according to particular instrument manufacturer recommendations.

a. Records of nitrogen generator maintenance and repairs will be maintained in the equipment log.

3.10 pH METERS

3.10.1 Calibration of pH meters will be done prior to use. Specific calibration procedures will depend on the individual meter. Acceptability of calibration will depend upon tolerances defined for each meter but in general pH measurements should be within ± 0.1 units of the reference buffer.

3.10.2 Maintenance of pH meters will include the following.

3.10.2.1 pH probes will be rinsed with deionized water between each use.

3.10.2.2 Contact of pH probe with protein-containing samples and biological samples will be minimized.

3.10.2.3 pH probes will be stored according to manufacturer recommendations.

3.10.2.4 pH probes will be cleaned and the reference solution replaced every three months, following manufacturer instructions

3.10.2.5 Reference buffers will be used which bracket the pH range being measured.

3.10.2.6 Reference buffers will not be used past manufacturer expiration dates.

3.10.2.7 Reference buffers will be dispensed into separate containers for measurement and never placed back into the source container.

3.10.3 Intermediate checks are not required for pH meters. Once calibration has been performed the pH meter is suitable for use for the remainder of the business day.

3.10.4 Records of calibration will be noted on the pH Meter Equipment Log. Any repair records will be maintained in the equipment log.

3.10.5 For most measurements of pH, the use of indicating pH paper is a suitable substitute for a pH meter.

3.11 PIPETTES

3.11.1 Calibration of pipettes will be performed annually, either by the Laboratory or by an approved calibration service provider. Pipettes refer to fixed volume, adjustable volume, multi-channel and repeater pipettes which employ

disposable pipette tips. Calibration service may be more frequent if normal operational use identifies the need.

3.11.2 Maintenance of pipettes will include the following.

3.11.2.1 Pipettes will be kept clean and stored in an upright position.

3.11.2.2 Pipettes will be disinfected prior to being sent out for calibration.

3.11.3 Performance verification of pipettes will be conducted when new pipettes are received in the Laboratory and on an intermediate basis if performance is suspect.

3.11.4 Records of pipette calibration, performance verification, parts replacement and repair will be maintained in the equipment log.

3.12 REFRIGERATORS AND FREEZERS

3.12.1 Refrigerators and freezers will not be calibrated but their proper operation will be monitored on a continual basis through the use of temperature logs. Temperature logs will be maintained on or in close proximity to each refrigerator or freezer.

3.12.2 Refrigerator and freezer maintenance will include the following

3.12.2.1 Temperatures will be monitored and recorded at least tri-weekly.

3.12.2.2 Door seals will be kept clean and replaced if leaking or damaged.

3.12.2.3 Freezers will be defrosted as needed to prevent excessive build-up of ice.

3.12.2.4 Refrigerator temperatures should read between 2 and 8°C. Freezers should read between -2 and -10°C.

3.12.3 If temperatures are noted to be just outside acceptable ranges then the thermostat may be adjusted to meet tolerances. If temperature control completely fails then all evidence, reference standards, quality control samples, drug standards or other temperature-sensitive materials are to be immediately re-located to a properly functioning refrigerator or freezer. The malfunctioning equipment is to be tagged as out of service and a repair request begun.

3.12.4 Records of thermostat adjustment, repairs and archived temperature logs are to be maintained in the equipment log.

3.13 THERMOMETERS

3.13.1 Thermometers used to check critical temperatures in the laboratory will be NIST traceable or will have their measurement accuracy verified against NIST traceable thermometers.

3.13.2 Thermometers may be of the digital type or of the graduated tube, liquid expansion type. Alternative thermometers for non-critical temperature

measurement may be used, such as bi-metal thermometers used in heating blocks. Refer to the individual equipment QA description for details on intermediate temperature checks.

3.13.3 Measurement accuracy will be verified at least annually for all thermometers used to check critical temperatures in one of the following ways.

3.13.3.1 Comparison to a NIST traceable, calibrated reference thermometer with comparative temperature tolerance of ± 2°C.

3.13.3.2 Certification by an approved calibration service provider.

3.13.4 Any thermometer found to be outside of measurement tolerance will be re-calibrated or replaced.

3.13.5 NIST traceability is good for two years from the date of purchase of a thermometer. Any certificate of calibration or traceability that accompanies a thermometer purchase should be retained.

3.13.6 Records of NIST traceability, certification by approved calibration service provider, intermediate temperature checks, annual temperature verification and repairs will be maintained in the equipment log.

3.14 TRACEABILITY

3.14.1 Analytical measuring equipment will be chosen that can show traceability to the International System of Units (SI) through an unbroken chain of comparisons to reference standards or primary standards.

3.14.2 Traceability to SI units is satisfied provided that the calibration supplier is accredited to ISO/IEC 17025:2005 and their scope of accreditation is relevant to the equipment being calibrated.

4 REAGENTS AND CONSUMABLE SUPPLIES

This chapter describes the receipt, preparation, verification, labeling and documentation associated with reagents and consumable supplies used by the Laboratory. The term 'reagent' refers to those chemicals either used as received or prepared by Laboratory personnel for use in technical procedures.

This chapter does not apply to the use of reference standards or reference materials which are employed as or used to prepare calibrators, positive quality control samples, stock or working drug standards, and are not used to calibrate, provide qualitative identification or verify quantitative accuracy. Forensic Scientists are directed to seek guidance from Supervisors and Management for specific topics not addressed in this or other related documents.

Deviations from these guidelines must be approved by the State Toxicologist, Laboratory Manager or the QA Manager and the approval documented.

4.1 REAGENTS

4.1.1 A reagent is broadly defined as a chemical, dilution of a chemical or combination of chemicals that is employed by the laboratory as specified in a technical procedure. Examples of reagents may include any of the following.

4.1.1.1 A dry chemical such as the salt sodium chloride

4.1.1.2 A liquid chemical such as the solvent ethyl acetate

4.1.1.3 A liquid chemical prepared from either a dry or liquid chemical such as a buffer or dilute acid for example sodium phosphate buffer or 0.1 M acetic acid.

4.1.1.4 A pre-diluted or pre-mixed chemical specified for use on a particular piece of equipment or by the manufacturer of a particular testing system for example a labeled antibody or antigen complex used for immunoassay testing

4.1.2 Receipt of reagents will include verification of the identity, quantity and, where applicable, the grade or purity of the order.

4.1.2.1 The person receiving the reagent should indicate the following on the packing slip; the date received, a check-mark by the items received to indicate the appropriate item and quantity were shipped, and receiver's initials or signature to indicate approval.

4.1.3 If a reagent has been defined as critical then it may only be obtained from approved suppliers. An approved vendor list will contain information on critical supplies and services obtained from pre-approved sources.

4.1.4 Reagents will be handled, transported and stored with consideration of the hazards associated with the reagent and any manufacturer recommendations.

4.1.5 Verification of reagents or checking the reliability of reagents will occur

through the normal testing process unless specified otherwise. If a reagent has been defined as a critical supply then the verification or reliability testing will occur before it is employed for evidence testing.

4.1.5.1 Acceptability of a reagent will be demonstrated through the attainment of acceptable results in routine casework whether they are proper qualitative identification of a known analyte or quantitative accuracy of a positive QC sample(s).

4.1.5.2 If verification or reliability checks do not support the reagent's use, then the reagent will be removed from service and any necessary retesting will be conducted.

4.1.6 The reagent container will be labeled as follows or the information will be recorded in a log that is referenced to the specific reagent.

3.1.6.1 Identity

4.1.6.1 Date of receipt, date of preparation or lot number

4.1.6.2 The following will be recorded where applicable.

a. Date the reagent container was opened
b. Initials of the person opening the container
c. Initials of the person who prepared the reagent
d. Raw material lot numbers used in reagents prepared in the laboratory
e. The date the reagent was verified or the reliability was checked
f. The initials of the person performing verification
g. The expiration date
h. Verification data, if performed

4.1.7 When reagents are transferred to secondary containers, the identity, date of preparation or lot number, initials of the person transferring and expiration date will be recorded on the container. For pure solvents transferred directly from supplier stock to a secondary container (e.g. oxford dispenser), labeling of the container with the solvent identity is sufficient.

4.1.8 Reagents prepared by the Laboratory may be stored at room temperature and used for up to 2 years after the preparation date unless specified otherwise. This timeframe may be shortened based upon reagent performance.

4.1.9 Unless specified otherwise, pure solvents do not have an associated expiration date.

4.1.10 Reagents that are prepared in the laboratory will be done so following good laboratory and safety practices.

4.2 CONSUMABLE SUPPLIES

4.2.1 A consumable supply is any material other than a reagent which is purchased for laboratory use. It is not equipment or instrumentation but may

be a component of either. For example, a GC injector inlet liner is used on the instrument as a consumable.

4.2.2 Consumable supplies should be selected through careful consideration of level of quality and performance and cost.

4.2.3 Any consumable supply that has been defined as critical will be purchased exclusively from an approved vendor as described on an approved vendor list.

4.2.4 As with reagents, receipt of consumable supply orders will be checked to verify that the quantity and type received is correct. See 4.1.2.1 above.

5 BLANK BLOOD CERTIFICATION

This chapter describes preparation and certification of blank blood and maintenance of the associated records. Forensic Scientists are directed to seek guidance from Supervisors and Management for specific topics not addressed in this or other related documents.

Deviations from these guidelines must be approved by the State Toxicologist or the QA Manager and the approval recorded in the blank blood testing documentation.

5.1 PREPARATION

5.1.1 Blank blood is obtained as packed blood cells from a blood bank, medical facility or other source, of human or animal origin.

5.1.2 Transfer the packed cells (one unit) into a container, add 10 g sodium fluoride and bring to a volume of 1 L with saline solution.

5.1.2.1 Saline Solution Preparation

a. In a glass or plastic container, dissolve 8.5 g sodium chloride in 250 mL deionized water (DI H_2O). Dilute to a volume of 1 L with DI H_2O. Volume may be adjusted provided that proportions remain constant.

5.1.3 The blank blood container is labeled with the following.

5.1.3.1 Identity

5.1.3.2 Lot number (YYBBxxxx)

5.1.3.3 Date of preparation

5.1.3.4 Initials of person preparing

5.1.4 Any transfers of the blank blood to separate containers must have the information described in 4.1.7 affixed to the containers.

5.2 CERTIFICATION

5.2.1 Blank blood is certified by testing it alongside case samples (or in the same manner as case samples) for those assays commonly used in the Laboratory.

5.2.2 When blank blood is used for an assay that is not commonly used in the Laboratory, it is good practice to include a sample of the blank blood as a matrix blank in the batch.

5.2.3 The lot number will be used to identify the blank blood on all certification test reports.

5.2.4 If the results of certification testing are negative then the blood is certified and suitable for use.

5.2.5 The presence of low levels of ubiquitous compounds such as caffeine, nicotine or cotinine is acceptable when certifying blank blood.

5.2.6 Store the blank blood in a refrigerator at 2° C to 8° C. It may be used for up to one year. Alternatively, the blood may be frozen and then later thawed. Frozen blank blood may be used for up to one year after the thaw date.

5.3 RECORDS

5.3.1 All records of blank blood certification testing are to be collected, briefly summarized by the preparer and submitted to and maintained by the QA Manager or designee.

6 REFERENCE MATERIALS

This chapter describes the purchase, handling and documentation related to reference materials used in the Laboratory. The term "reference material", for the purpose of this chapter and its application to toxicology testing, is synonymous with a drug, drug metabolite, toxin, or any other substance which is the focus of toxicological calibration, value assignment or quality assurance. Forensic Scientists are directed to seek guidance from Supervisors and Management for specific topics not addressed in this or other related documents.

Deviations from these guidelines must be approved by the State Toxicologist or the QA Manager and the approval recorded in the receipt records or other location, where appropriate.

6.1 DEFINITIONS

6.1.1 Certified Reference Material (CRM): Reference material, accompanied by documentation issued by an authoritative body and referring to valid procedures used to obtain a specified property value with uncertainty and traceability. [6.6.1]

6.1.2 Reference Material (RM): Material, sufficiently homogenous and stable regarding one or more properties, used in calibration, in assignment of a value to another material, or in quality assurance. [6.6.1]

6.2 PURCHASE

6.2.1 RM's are considered critical supplies and will be purchased only from suppliers who have been evaluated and approved as vendors of critical supplies. An approved reference material supplier will have a Vendor Evaluation and Approval on file with the QA Manager.

6.2.2 Approval of a reference material provider is based on adherence to ISO 17025 or ISO Guide 34 requirements, accreditation to other recognized and appropriate requirements, sole-source justification, ability to provide traceability to SI units, or a combination of these.

6.2.3 An RM may be obtained from a non-approved supplier if that supplier is the sole source of the RM; such as the case with a patented or proprietary compound.

6.2.4 Wherever possible, the highest quality and highest purity reference materials appropriate to their intended use will be purchased by the Laboratory. Lower purity reference materials may be employed but all necessary steps must be taken to compensate for sub-optimal purity (examples: factor purity into calculations, examine the effect, if any, of impurities on testing).

6.2.5 Upon receipt of RM's, the purchasing documents will be inspected to verify the following:

6.2.5.1 The identity.

6.2.5.2 The supplier is currently listed as an approved vendor of RM. (see exception in 6.2.3)

6.2.5.3 The RM is of the correct type, purity or grade as specified in the original order documentation.

6.2.5.4 The quantity received is correct.

6.2.5.5 The person receiving the material should indicate the following on the packing slip; the date received, a check-mark by the items received to indicate the appropriate material and quantity were shipped, and receiver's initials or signature to indicate approval.

6.2.6 Copies of any certificates of analysis (COA's), traceability or property value uncertainty will be retained and filed appropriately.

6.3 LABELING AND STORAGE

6.3.1 Reference materials will be labeled with, at a minimum, the identity and date of receipt.

6.3.2 Reference materials will be stored securely, to maintain their integrity, and according to any manufacturer recommended storage conditions and applicable laws.

6.3.3 Powdered reference materials will be stored at room temperature unless otherwise specified.

6.3.4 Liquid reference materials will be stored in a freezer at temperatures between -2°C and -10°C. If such temperatures are incompatible with the freezing point of the material, they will be stored refrigerated between 2°C and 8°C.

6.4 VERIFICATION

6.4.1 Reference materials obtained from approved suppliers do not require verification as long as a COA (however named) is available to document purity analysis and the method(s) of identification. The COA may describe the traceability, purity, certification testing, manufacture, quality, shelf life, lot number, production run or other information that supports the use of the material in the laboratory.

6.4.2 When reference materials are obtained from a non-approved supplier, the identity and purity of the material will be examined prior to use.

6.4.2.1 Acceptable means of reference material testing include gas chromatography, high performance liquid chromatography, mass spectrometry or other measurements of physical constants.

6.5 RECORDS

6.5.1 COA's (however named) will be retained for each reference material received. If a reference material purchase is a resupply of a previously

purchased lot number for that material, then duplicate certificates do not need to be retained.

6.5.2 Records of any reference material verification will be retained with COA's. The verification records will identify the person performing the verification and the date of verification.

6.5.3 Purchasing documents will be maintained by office administrative staff.

6.6 REFERENCES

6.6.1 International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM), 3rd ed. BIPM/IEC/IFCC/ISO/IUPAC/IUPAP/OIML, International Organization for Standardization (ISO), 2006.

7 STANDARD SOLUTION PREPARATION

This chapter describes the preparation, labeling, storage and verification of standard solutions and maintenance of records associated with the same. The terms "standard solution" and "standard" are synonymous for the purpose of this chapter and its application to toxicology testing. Forensic Scientists are directed to seek guidance from Supervisors and Management for specific topics not addressed in this or other related documents.

Deviations from these guidelines must be approved by the State Toxicologist or the QA Manager and the approval recorded in the solution preparation documents or other location where appropriate.

7.1 DEFINITIONS

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

7.1.2 Calibrator: A preparation of a standard in biological material to a known concentration that is used to define a concentration-response relationship, typically through the use of a calibration or standard curve.

7.1.3 Stock Standard: An initial preparation of a standard at a known concentration either through the dilution of a solid reference material or purchased as a pre-diluted solution.

7.1.4 Working Standard: Subsequent dilutions of a stock standard or collection of stock standards for direct use in the preparation of calibrators or controls.

7.2 PREPARATION

7.2.1 Standard solutions are prepared using appropriately selected reference materials which have been sufficiently characterized. This may include descriptions of material purity, degree of hydration, salt form, free-base form or other information.

7.2.2 The reference materials should be purchased from approved suppliers and stored appropriately as recommended by the supplier and described in the quality assurance guidelines.

7.2.3 Stock Standard Preparation

7.2.3.1 Stock standards will be prepared in either acetonitrile or methanol, whenever possible. The solvent may be varied based upon considerations of stability or solubility of the reference material.

7.2.3.2 Solid reference material will be weighed on a 5-place or better analytical balance which has been calibrated by an approved service provider that can show traceability to SI units. The balance calibration will be checked by ASTM Class 1 weights before reference material is weighed.

a. The balance calibration check will be conducted each time a standard is weighed or daily, whichever is least. Four weighings will be made using 1.0, 0.1, 0.01 and 0.005 g masses. The weighings must read within ±1.0% of the target mass. If this tolerance is not met then the QA Manager must be notified.

b. Balance calibration checks are documented on Standard Solution Preparation Records, copies of which are filed alphabetically in the Balance Calibration Check Log of the balance equipment log.

7.2.3.3 The amount of reference material to be measured is calculated based on the desired standard solution concentration, the volume of solution required, the purity of the reference material, the molecular weight of the compound and the molecular weight of the compound salt or hydrate, where applicable.

7.2.3.4 The amount of reference material to weigh is determined from the following equation:

$$Weight_{RM} = target\ weight \times \left[\frac{MW_{Salt/Hydrate}}{MW_{Parent}}\right] \times \left[\frac{1}{\%Purity}\right]$$

a. Percent purity is purity divided by 100. Manufacturer purity of 98% or more will use a percent purity of 1.00.

b. Consult the reference material's certificate of analysis (however named) or other supplier information for purity and composition details.

c. Record the actual weight of the reference material on the Standard Solution Preparation Record.

7.2.3.5 Transfer the weighed reference material into the appropriately sized Class A volumetric flask and fill to volume with the selected solvent. Record the serial number of the flask on the Standard Solution Preparation Record.

7.2.3.6 The final concentration of the stock standard is determined from the following equation:

$$Concentration = \left[\frac{Quantity\ Weighed}{Flask\ Volume}\right] \times \left[\frac{MW_{Parent}}{MW_{Salt/Hydrate}}\right] \times [\%Purity]$$

7.2.4 Commercially obtained stock standards purchased as pre-diluted solutions may be used without further preparation.

7.2.5 Working Standard Preparation

7.2.5.1 Instructions for the preparation of working standards are contained in individual technical procedures. Storage and labeling of working standards will be the same as those for stock standards.

7.3 LABELING

7.3.1 Standards will be labeled with the following information:

7.3.1.1 Identity

7.3.1.2 Concentration

a. If a standard contains multiple compounds at different concentrations, the container does not need to list each concentration, provided this is described in the relevant test procedure or preparation documentation

7.3.1.3 Preparation Date

7.3.1.4 Expiration Date

7.3.1.5 Solvent

7.3.1.6 Initials of Preparer

7.3.2 If labels become smeared or otherwise difficult to read, they will be replaced using the original Standard Solution Preparation Record as reference.

7.3.3 If the standard is transferred to a new container or split between several containers they shall all contain the information listed in 7.3.1.

7.4 STORAGE

7.4.1 Any standard solution prepared in the laboratory will be stored in amber-colored bottles labeled with the information described in 7.3.1.

7.4.2 Standards purchased as pre-diluted solutions may be stored in their original containers. These containers should be labeled to contain, at a minimum, the identity, concentration, manufacturer lot number, solvent and expiration date.

7.4.3 Standard solutions should be stored in freezer temperatures between -2°C and -10°C. If these temperatures are below the solvent's freezing point then they may be stored in refrigerated temperatures of between 2°C and 8°C.

7.4.4 Standard solutions shall not be stored at room temperature unless specified by a supplier as necessary to maintain stability.

7.5 VERIFICATION

7.5.1 It is not necessary to verify the identity or concentration of stock standards.

7.5.2 Working standards will undergo verification of both the composition and concentration of the standard. That verification can be accomplished by any of the following ways.

7.5.2.1 Addition of the working standard to a biological matrix and testing as a specimen.

7.5.2.2 Comparison of the working standard to an existing, comparable standard; this may involve a biological matrix or may not.

7.5.2.3 Testing of the working standard used as a calibrator or positive QC sample in batch analysis. This is usually reserved for situations where there is no pre-existing, comparable standard available for comparison.

7.5.3 Single-compound working standards that are not used in regularly-performed testing do not require verification, provided they are prepared from certified reference materials accompanied by a certificate of analysis.

7.6 RECORDS

7.6.1 The preparation of standard solutions from solid reference materials will be documented on the Standard Solution Preparation Record.

7.6.1.1 A copy of this record will be added to the Balance Calibration & Calibration Check binder if it was used to document the balance calibration check.

7.6.1.2 The original record will be filed alphabetically in the Standard Solution Preparation Binder.

7.6.2 The preparation of standard solutions from purchased certified reference materials (e.g. 1.0mg/mL methanolic standards) or stock solutions prepared in-house, will be documented on the Solution Preparation Worksheet.

7.6.2.1 Preparation and verification documentation for standards prepared for use in regularly-performed testing will be retained by the QA Manager or designee

7.6.2.2 Solution Preparation Worksheets for single-compound working standards that are not used in regularly-performed testing will be filed alphabetically in the Standard Solution Preparation Binder.

8 PROFICIENCY TESTING

This chapter describes the TLD's proficiency testing program for Forensic Scientists. Forensic Scientists are directed to seek guidance from Supervisors and Management for specific topics not addressed in this or other related documents.

The objectives of the proficiency testing program are to demonstrate the current competence of the analyst and the Laboratory, ensure that quality work is being maintained, identify areas where additional training or resources would be beneficial, and verify the validity of technical procedures.

Deviations from these guidelines must be approved by the State Toxicologist or the QA Manager and the approval recorded in the proficiency test record or other location where appropriate.

8.1 DEFINITIONS

8.1.1 Approved test provider: An external proficiency test (PT) provider that has been evaluated and found to comply with the standards of an accrediting body (ASCLD/LAB) or found to meet the proficiency test needs of the laboratory.

8.1.2 Assigned value: The value attributed to a particular property of a proficiency test item.

8.1.3 Blind proficiency test: The analyst is not aware that they are performing a proficiency test.

8.1.4 External proficiency test: A test provided by a source external to the laboratory.

8.1.5 Internal proficiency test: A test supplied by the testing laboratory.

8.1.6 Open proficiency test: The analyst is aware of the nature of the proficiency test.

8.1.7 Proficiency review committee (PRC): A committee of individuals appointed by the Board of ASCLD/LAB, because of their experience and expertise, to provide oversight for ASCLD/LAB in the proficiency testing program for specific forensic disciplines.

8.1.8 Proficiency testing: Evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons.

8.1.9 Proficiency test item: Sample, product, artifact, reference material, piece of equipment, measurement standard, data set or other information used for proficiency testing.

8.1.10 Proficiency testing provider: Organization which takes responsibility for all tasks in the development and operation of a proficiency testing scheme.

8.1.11 Proficiency testing scheme: Proficiency testing designated and operated in one or more rounds for a specified area of testing, measurement, calibration or inspection.

8.2 FREQUENCY

8.2.1 Annually, each Forensic Scientist shall successfully complete at least one proficiency test for ethanol in blood or serum and one proficiency test for drugs in one type of specimen.

8.2.2 External proficiency test providers will typically provide a delivery schedule for the year's testing cycle.

8.3 APPROVED TEST PROVIDERS

8.3.1 The following proficiency test providers are approved for use for the indicated proficiency testing schemes.

8.3.1.1 Collaborative Testing Services (CTS)

a. 564 – Blood alcohol analysis

8.3.1.2 College of American Pathologists (CAP)

a. AL1 – AACC/CAP Alcohol/Ethylene Glycol/Volatiles
b. FTC – Whole Blood Forensic Toxicology
c. LN14 – Whole Blood Ethanol Calibration Verification/Linearity
d. T – Toxicology

8.3.1.3 Wisconsin State Laboratory of Hygiene (WSLH)

a. AL – Legal Alcohol

8.4 GENERAL

8.4.1 External proficiency tests will only be obtained from suppliers who have been approved by the State Toxicologist or their designee.

8.4.2 Internal proficiency tests will only be produced by the QA Manager or their designee.

8.4.3 Proficiency test items will be retained by the laboratory until a summary report is received and any corrective actions satisfactorily completed.

8.4.4 Proficiency test results will be retained according to the record retention schedule and for at least the duration of a single accreditation cycle. Proficiency test records may include:

- Proficiency test unique identifier
- How tests were obtained or created
- Written instructions for completion
- Identity of person taking the test
- Due date and submission date

- Copy of the proficiency answer sheet(s)
- Copy of the proficiency test evaluation form
- Any discrepancies noted
- Details of corrective actions taken (when necessary)

8.4.5 Where possible, proficiency testing will be conducted in a manner that is consistent with standard testing methods used in the laboratory. If the test provider instructions conflict with laboratory practice, the test provider instructions are authoritative. For example, standard laboratory practice may include volatile testing but this is unnecessary if stated in test provider instructions or if test material is lyophilized.

8.4.6 Proficiency testing will be of the open variety.

8.4.7 The QA Manager is responsible for the operation of the proficiency testing program.

8.5 ASSIGNMENT AND SCHEDULING

8.5.1 The QA Manager or their designee will coordinate the ordering, receipt and assignment of proficiency tests.

8.5.2 The QA Manager or their designee will provide copies of PT paperwork to the analyst including directions on the handling or preparation of the PT item and the reporting paperwork.

8.5.3 Once in receipt of their assigned test, the analyst will make every effort to complete testing and submit results in advance of the deadline for submission of test results. This will allow for review of the results and assignment of supplemental testing where appropriate.

8.5.4 If the PT cannot be completed by the deadline, the reason will be documented in the PT file and follow-up testing of the PT item may be conducted after the deadline has elapsed. The production of an acceptable PT result after the assigned value has been reported by the PT provider is not considered a successful completion of a PT.

8.6 TESTING PROTOCOL

8.6.1 Analysts will conduct those tests appropriate to the nature of the proficiency test and may take into account any case scenario or history included by the PT provider.

8.6.2 Any special instructions for testing, such as storage conditions or timetables for PT item stability, shall be followed by the analyst.

8.6.3 Generally, the PT will be completed using current laboratory test methods. Where a test method is not available but suitable reference materials are, a test method may be developed and utilized for the PT. The decision to report the PT results from a novel test method rests with the QA Manager.

8.6.4 The analyst will report quantitative results in accordance with any specific laboratory reporting guidelines concerning rounding, truncation or significant figures.

8.6.5 When the PT provider requests quantitative results in units different that those reported by the laboratory, the analyst will convert their results to the requested units.

8.6.6 The analyst will submit all data and reporting paperwork to the QA Manager upon completion of the PT.

8.6.7 The QA Manager or their designee will authorize or release PT results to the PT provider.

8.7 REVIEW OF PERFORMANCE

8.7.1 The PT provider will publish a report of the results for an individual PT cycle. That report may indicate the statistical evaluation of all participant results with a separate evaluation of the analyst's results.

8.7.2 The assigned value for a PT item, unless otherwise specified by the PT provider, will be assumed to be the participant mean/average, and the standard deviation for the proficiency assessment (SDPA) will be assumed to be the standard deviation of the participant results. Unless otherwise specified, it is assumed that participant results have been examined for outlier data which have then been removed.

8.7.3 A PT is considered successful if it meets the evaluation criteria of the PT provider. When the PT provider does not provide performance criteria, the QA Manager may evaluate the results in terms of standards of accuracy within the Forensic Toxicology community or by the use of performance statistics.

8.7.4 A collection of performance statistics may be calculated to evaluate the analyst's reported PT results. One or more of the commonly used statistics for quantitative results may be used.

8.7.5 The percent difference, also known as relative standard error, is calculated according to the following equation; where $D_{\%}$ is the percent difference; x is the analyst's result; and X is the assigned value. In forensic toxicology, acceptable percent differences of ±20% for drugs and ±10% for alcohol are commonly used.

$$D_{\%} = \frac{(x - X)}{X} \times 100$$

8.7.6 Satisfactory Proficiency Results

If the test results are satisfactory, the QA Manager will complete documentation of the satisfactory result in the records. Notification of satisfactory completion will be issued to the analyst and the Supervisor in writing. The analyst and their Supervisor will document their review of the analyst's performance by initialing and dating the written notification. The

notification is then reviewed by the Laboratory Director, with the review documented by initialing and dating the written notification.

8.7.7 Proficiency Test Discrepancies

If there is a discrepancy between the analyst's test results and the provider's results, the QA Manager will immediately notify the analyst who performed the test and their Supervisor. The QA Manager and the Laboratory Director will determine a course of action, if necessary, and coordinate that process with the PRC.

If an analyst's performance on a proficiency test requires further development to meet quality standards, the QA Manager will work with the Supervisor and the analyst on a plan of action which may include removal of the analyst from work and participation in remedial training. The QA Manager will prepare a report to the Laboratory Director, outlining the issues and the actions taken.

The proficiency test records will contain a record of the discrepancy between the analyst's test results and those of the test provider. The QA Manager will retain the PT records for the Laboratory.

8.7.8 Proficiency Testing and Job Performance

Any problems identified from the review of a proficiency test, if reflective of difficulties with an analyst's individual work performance, will be addressed by the Supervisor and documented in the supervisory desk file. The Supervisor may enlist input and assistance from the TLD management, and other appropriate individuals.

8.8 REFERENCES

8.8.1 EURACHEM, Selection use and interpretation of proficiency testing (PT) schemes, 2nd Ed. 2011

8.8.2 EuroLab, Technical report No. 1/2007 Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation, March 2007

8.8.3 ISO 13528:2005, Statistical methods for use in proficiency testing by interlaboratory comparisons

8.8.4 ISO/IEC 17042:2010, Conformity assessment – General requirements for proficiency testing

8.8.5 M. Thompson, S.L.R. Ellison and R. Wood, The international harmonized protocol for the proficiency testing of analytical chemistry laboratories, Pure Appl. Chem. 78(1): 145-196 (2006).

9 VALIDATION PROCEDURE FOR CONFIRMATORY METHODS

9.1 POLICY

It is the policy of the TLD to employ methods that meet the needs of the customer and are appropriate for the testing being performed. In the absence of specifically designated methods by a customer, the Laboratory policy is to select methods published in international, regional or national standards, relevant scientific texts or journals, equipment manufacturer-specified methods or laboratory-developed methods. When laboratory-developed methods are selected, they are to be appropriate for the intended use and validated.

The validation will be as extensive as necessary to meet the needs of the given application and the results of the validation will be recorded along with the procedure used for validation and the fit for use of the method. The range and accuracy of values obtained from validated methods will be relevant to the customers' needs and shall include considerations of detection limits, selectivity, linearity, repeatability, robustness and stability.

Any adjustments or deviations from the procedures below must be approved by the State Toxicologist or the QA Manager, and appropriately documented in the validation records.

9.2 PURPOSE

This chapter defines the procedures for method validation when using chromatographic instrumentation to confirm the identity and determine the concentration of drugs and/or metabolites. This procedure applies to methods utilizing gas chromatography (GC), high performance liquid chromatography (LC), and combined GC and LC mass spectrometry (MS) including: GC/MS, LC/MS, GC/MS-MS, and LC/MS-MS.

9.3 PRINCIPLE

Method validation is a part of the Laboratory's overall quality control and assurance program. Method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use. Proper validation is essential for defining the parameters within which an analytical technique can be applied and it is mandatory for all confirmatory procedures that are frequently performed.

Validation of confirmatory methods includes documentation of the performance limits of the particular assay, records of which are maintained by the QA Manager. Typical validation characteristics should be recorded to demonstrate a method's theoretical and/or empirical limits of detection and quantitation, its dynamic range, precision, accuracy, selectivity, stability and robustness.

9.4 DEFINITIONS (SEE REFERENCES)

9.4.1 Accuracy: The degree of closeness of the determined value to the nominal or known true value under prescribed conditions. This is sometimes termed *trueness*.

9.4.2 Blank: A sample of a biological matrix to which no analytes have been added that is used to assess the selectivity of the bioanalytical method.

9.4.3 Dynamic (quantification) range: The range of concentration, including ULOQ and LLOQ that can be reliably and reproducibly quantified with accuracy and precision through the use of a concentration-response relationship.

9.4.4 Intermediate precision: Obtaining the magnitude of a particular property of a sample more than once by keeping global factors, other than time, constant. Alternatively known as between-run precision.

9.4.5 Limit of detection (LOD): The lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise, or the lowest concentration that can be detected but not quantified. The limit of detection and lower limit of quantitation shall never be the same value.

9.4.6 Linearity: The ability of the method to produce results which are directly proportional to analyte concentration within a given range.

9.4.7 Lower limit of quantification (LLOQ): The lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

9.4.8 Precision: The closeness of agreement (*degree of scatter*) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

9.4.9 Repeatability: Obtaining the magnitude of a particular property of a sample more than once by keeping global factors (analyst, procedure, instrument, laboratory and day) constant. Alternatively known as within-run precision.

9.4.10 Robustness: Evaluation of constancy of results when internal factors, whether instrumental (flow rate, injection volume, column temperature, etc.) or in the extraction procedure (strength of buffer, acids, bases, duration of mixing, etc.) are deliberately varied.

9.4.11 Selectivity: The ability of the bioanalytical method to measure and differentiate the analytes in the presence of components that may be expected to be present. These could include metabolites, impurities, degradants, or matrix components.

9.4.12 Stability: The chemical stability of an analyte in a given matrix under specific conditions for given time intervals.

9.4.13 Standard curve: The relationship between the experimental response value and the analytical concentration (also called a *calibration curve*).

9.4.14 Upper limit of quantification (ULOQ): The highest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

9.4.15 Validation: The confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.

9.5 GUIDANCE

9.5.1 Validation of a method is preceded by method development wherein a general knowledge of the method's performance would have already been estimated. Validation is not a substitute for proper method development; it is a part of the formalization of a method.

9.5.2 The laboratory recognizes a clear distinction between a method's performance limits for a particular analyte and the analyte's potential concentration in a given specimen. The validation protocol is intended to establish the method's reliability and reproducibility and may not necessarily cover the entire concentration range possible for the analyte in a given sample.

9.5.3 Wherever possible, the laboratory will conclude validation of a confirmatory, chromatographic method prior to implementing that method. However, in the case of a novel or infrequently encountered analyte, the laboratory may forego this process to expedite release of results. In these instances the laboratory will maintain the highest possible degree of quality control during the testing phase. The extent of validation will depend on such constraints as estimated frequency of method employment, time and customer requirements. Due to these constraints, not all validation characteristics will be applied in all situations but all characteristics which are applied will be documented and objective evidence recorded and maintained.

9.5.4 Validation should proceed according to the documented method that has been developed. Alterations in the method that are necessitated during validation will require recommencement of all or part of the validation procedure. (Example: A ULOQ value of 2.0 mg/L is established and, during the course of measuring precision, the 2.0 mg/L coefficient of variation is found to exceed ±15%.)

9.5.5 The simplest model that defines the concentration-response relationship should be used. Selection of weighting factors or the use of non-linear regressions should be justified and that justification documented.

9.5.6 The order in which specific validation characteristics are determined is left to the discretion of the scientist.

9.6 CRITERIA FOR ACCEPTANCE

9.6.1 All monitored chromatographic peaks should be present and have a symmetrical appearance.

9.6.2 The retention times (t_R) of analyte peaks should be within ±2% of the t_R of the calibrator(s), controls or other standard specified in the procedure. In the case of liquid chromatography, larger retention time deviations may be acceptable, particularly when gradient separations are employed. In those instances the t_R should not vary by more than ±5% from the standard established in the method.

9.6.3 For mass spectrometry detection in selected ion monitoring mode, the analyte ion ratios should be within ±20% of the ratios measured for the calibrator(s). Permissible ion ratios for LC/MS are ±25%. Ion ratio tolerances also apply to any internal standard.

9.7 DYNAMIC RANGE

For a multi-point calibration, one of the criteria for acceptance is usually the correlation coefficient. For most applications, an acceptable correlation coefficient is 0.99. However, there may be circumstances where a correlation coefficient of 0.98 is minimally acceptable. In addition, it is good practice to evaluate the range of the calibration by calculating the value of each calibrator against the curve. Values of ±20% are generally acceptable for most applications, although ±10% are preferred for analytes such as ethanol. Single point calibrations are discouraged unless controls are used at or close to the ULOQ and LLOQ. [9.19.2]

1. Prepare a minimum of 6 calibration points to make a calibration curve. The standards should encompass the expected dynamic range and include the expected LLOQ.
2. Include a blank specimen and a negative control.
3. Complete the analysis.
4. Generate a standard curve using only those points that result in a correlation coefficient of 0.99 or better.
5. All points in the dynamic range must meet the criteria for identification and acceptance.
6. Repeat the procedure on 10 separate days to verify the correlation between response and concentration. [Note: This can be done when performing between-day precision.]

9.8 LIMITS OF QUANTITATION

The lower limit of quantitation is defined as either the mean value (n=10) of the blank (X_m) plus ten standard deviations (LOQ=X_m + 10SD), or that concentration of an analyte in a sample which, when analyzed, meets all required criteria for identification and produces a quantitative value within ± 20% of the theoretical/expected value. [9.19.2]

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen and a negative control.
3. Prepare 5 concentrations near the expected LLOQ and 5 concentrations near the expected ULOQ.
4. Complete the analysis and generate the standard curve.

5. The lowest concentration that meets the criteria for identification and acceptance is the *presumptive*-LLOQ.
6. The highest concentration that meets the criteria for identification and acceptance is the *presumptive*-ULOQ.
7. Both *presumptive* LOQ's must be within the dynamic range of the method.
8. Following the determination of precision (described below) for the *presumptive* LLOQ and ULOQ, they are deemed the actual limits.

9.9 PRECISION

Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV. [9.19.2]

9.9.1 **WITHIN-RUN:** A measure of precision within a single batch or analytical run. Alternatively known as repeatability.

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen and a negative control.
3. Prepare 5 replicates of a minimum of 3 concentrations to include the LLOQ and the ULOQ.
4. Complete the analysis and generate the standard curve.
5. All replicates should meet the criteria for identification and acceptance. Deviations from this standard must be approved by the State Toxicologist or QA Manager.
6. Calculate the average, standard deviation, and CV for each concentration.
7. All concentrations should be within ±20% of their nominal values and the CV should be ≤15% for all controls except the LLOQ (≤20%).

9.9.2 **BETWEEN-RUN:** A measure of precision with time. Alternatively known as intermediate precision.

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen and a negative control.
3. Prepare a minimum of 3 concentrations to include the LLOQ and the ULOQ.
4. Complete the analysis and generate the standard curve.
5. All specimens should meet the criteria for identification and acceptance. Deviations from this standard must be approved by the State Toxicologist or QA Manager.
6. Repeat this procedure for a minimum of 10 days.
7. Calculate the average, standard deviation, and CV for each concentration.
8. Individual and average concentrations should be within ±20% of their nominal values and the CV should be ≤15% for all controls except the LLOQ (≤20%).

9.10 LIMIT OF DETECTION

The limit of detection is defined as either the mean value (n=10) of the blank (X_m) plus three standard deviations (LOD=X_m + 3SD), or that concentration of an analyte in a sample which, when analyzed, produces a signal-to-noise ratio of at least three but a concentration which is not lower than X_m+3SD. [9.19.2]

9.10.1 Theoretical

1. Using the blank specimens analyzed over 10 days for the between-run precision; determine the mean (X_m) and standard deviation (SD) value for the peak height or area at the t_R for the analyte.
2. Calculate the LOD: LOD=X_m+3SD

9.10.2 Experimental

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen and a negative control.
3. Prepare 5 samples at concentrations below the LLOQ.
4. Complete the analysis and generate the standard curve.
5. Identify the lowest concentration sample with a signal-to-noise ratio ≥3.

In general, the LOD shall be defined as the experimental value provided it is not less than the theoretical value.

9.11 ACCURACY

Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The mean value should be within 20%. The deviation of the mean from the true value serves as the measure of accuracy. [9.19.2]

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen and a negative control.
3. Prepare 5 replicates of a minimum of 3 concentrations to include the LLOQ and the ULOQ.
4. Complete the analysis and generate the standard curve.
5. All specimens should meet the criteria for identification and acceptance.
6. All concentrations should be within ±20% of their nominal values.

9.12 SELECTIVITY

Selectivity of the method to endogenous matrix components will be established through analysis of a minimum of 6 sources of the specific matrix. Selectivity of the method to xenobiotics and/or metabolites will be established through analysis of blank matrices containing compounds that may possibly result in interferences. The number of compounds and their concentrations should be selected to provide an

estimation of potential interferences which would reasonably be encountered during testing.

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen and a negative control.
3. Prepare a minimum of 6 sources of blank matrix. Previously tested specimens that would reasonably be expected to be free of the compound(s) being tested can serve as sources of blanks. Alternatively, if the blank specimens used during determination of limits, dynamic range, precision or accuracy were from separate sources, then the results of this testing can suffice for establishing matrix selectivity.
4. Prepare blank matrix samples containing xenobiotics. Document their type and concentration.
5. Complete the analysis and generate the standard curve.
6. Examine all blank matrices and fortified blanks for interference with the compound(s) being tested. Document any interferences noted during testing.

9.13 ROBUSTNESS

Robustness will be examined through deliberate variations in internal factors of the method. The variations are intended to be slight and serve as predictive measures of the effect of minor changes on the method. The number and degree of variations to be examined during validation are left to the scientist's discretion, but all variations and their resulting effect on the method will be documented in the validation record. Ideally the variations are made one at a time and assessed over the analysis of a batch. For testing robustness, the batch can be defined as the calibration curve, blank specimen, negative and positive controls. Examples of variations to consider may include the following:

- Carrier gas or mobile phase flow rate
- Mobile phase composition
- Mobile phase pH
- Column temperature
- Injection volume
- Injection temperature
- Split ratio
- Nebulizer pressure
- Drying gas temperature or flow
- Corona current
- Vaporizer temperature
- Concentrations of buffers, acids or bases used in extraction
- Duration of mixing, evaporation or derivatization

9.14 STABILITY

Stability may be assessed over conditions of long or short term storage, in light of particular storage containers, with respect to time or following freeze-thaw cycles. Amongst the stability test that may be performed are: freeze-thaw stability, sample container stability, short-term storage and processed sample stability.

9.14.1 Freeze-thaw stability

1. Prepare fresh samples of low and high concentrations of drug(s) in the specified matrix in triplicate. Prepare the samples at volumes and in containers which are typically received in the laboratory.
2. Freeze the samples under typical conditions for a 24-hour period and record the storage temperature.
3. Thaw the samples at room temperature and then re-freeze them for a minimum of 12 hours.
4. Repeat the thaw/re-freeze cycle two additional times and then thaw the samples for testing.
5. Test the samples as normal and record the results.

9.14.2 Sample container stability

1. Prepare fresh samples of low and high concentrations of drug(s) in the specified matrix in triplicate. Prepare the samples at volumes and in containers which are typically received in the laboratory.
2. Maintain the specimens at recorded temperatures and time periods that reasonably correspond to those encountered between collection, transport and delivery to the laboratory or, where possible, that correspond to estimates of long-term storage conditions.
3. Test the samples as normal and record the results.

9.14.3 Processed-sample stability

1. Prepare fresh samples of low and high concentrations of drug(s) in the specified matrix in triplicate.
2. Process/extract the samples according to the method and transfer the processed batch to the vessels used for analysis (example: autosampler vials).
3. Store the processed batch for a minimum of 12 hours or a standard work shift, whichever is longer, in a refrigerator. Record the storage temperature.
4. Bring the processed batch to room temperature, test as normal and record the results.

9.15 DILUTION SUITABILITY

1. Prepare a minimum of 6 standard points to make a standard curve. The standards should encompass the pre-determined dynamic range.
2. Include a blank specimen, negative control and positive control(s).
3. Prepare a minimum of 3 concentrations above the ULOQ (examples: 2x, 4x, 6x, 8x and 10x the ULOQ).
4. Prior to sample processing, dilute these specimens so that the diluted concentrations lie within the dynamic range of the method. Dilutions should be made using blank matrix but dilutions using buffer, dilute acids or bases, or deionized/distilled water may also be appropriate.
5. Complete the analysis and generate the standard curve.
6. Apply the individual multiplier values to each diluted specimen to calculate the final concentration.

9.16 MINIMAL REQUIREMENTS FOR VALIDATION

The minimal characteristics to be determined during method validation depend upon the intended use of the method. This should be decided at the outset of the validation process and will be based primarily, but not exclusively, on the expected frequency of use of the method.

For those methods that will be utilized very frequently by the Laboratory, it would be appropriate to determine method performance for all of the characteristics noted herein.

For frequently employed methods, at a minimum, the limits of detection and quantitation, dynamic range, precision, accuracy, and selectivity should be recorded. Between-day precision may be omitted as warranted.

For methods which are infrequently utilized, the limits of quantitation and dynamic range should be determined where possible. In the absence of formalized validation for infrequently utilized methods, the reporting of confirmed results shall be restricted to those established by the batch parameters and in keeping with the best practices of Forensic Toxicology.

9.17 VALIDATION FOLLOWING METHOD MODIFICATION

In the event that a frequently utilized method must be modified after validation, the effect of the modification on the established performance parameters of the method will be evaluated. A technical review of the modification will be conducted by senior technical staff and they will provide written recommendations of any required, additional validation testing. These additional recommendations may include full method validation depending upon the method modification.

9.18 VALIDATION SUMMARY/REPORT

At the conclusion of method validation a summary or report will be written. The summary or report will include a copy of the standard operating procedure followed during validation, any statistical calculations made in support of the validation findings and summaries of the results of all completed validation steps. The original data collected during validation will also be provided for examination.

The report supporting data will be submitted to the QA Manager, who will review the report to ensure compliance of the report findings with the QMS. The report, along with any additional information or recommendations by the Laboratory or QA Manager, will be submitted to the Laboratory Director, who is responsible for approving the method validation.

For infrequently employed methods, a validation report may not need to be produced.

9.19 REFERENCES

(The inclusion of references in this list does not indicate an endorsement, in whole or in part, of the contents of the reference by the WSP Toxicology Laboratory Division. The policies and procedures of the Division are considered authoritative in

any conflicts or scientific differences of opinion existing between Division procedures and those in the references.)

9.19.1 Guidance for Industry – Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, May 2001.

9.19.2 SOFT / AAFS Forensic Toxicology Laboratory Guidelines 2006 Version, Society of Forensic Toxicologists Inc. and the American Academy of Forensic Sciences, Toxicology Section, SOFT/AAFS Laboratory Guidelines Committee 2005-2006.

9.19.3 M. Thompson, S.L.R. Ellison and R. Wood, Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure Appl. Chem. 74 (5): 835-855 (2002).

10 PROCEDURE FOR THE GRAVIMETRIC CERTIFICATION OF HAMILTON MICROLAB 500A SERIES DILUTER DISPENSERS

10.1 POLICY

Each Hamilton Microlab diluter dispenser (a.k.a. diluter), will be certified by this procedure on an annual basis. If the diluter undergoes a manufacturer-provided repair which includes certification or if certification is conducted by the manufacturer or similarly approved service provider, then that certification will satisfy the requirement for that year. Any outside certification will be by an approved calibration provider that can show traceability to SI units.

Any adjustments or deviations from this procedure must be approved by the State Toxicologist or the QA Manager, and appropriately documented.

10.2 EQUIPMENT

10.2.1 Calibrated balance: Mettler XP205, or equivalent

10.2.2 Calibrated reference weight set (ASTM class 1)

10.2.3 Calibrated thermometer (0.1°C resolution or better)

10.2.4 Weighing vessels (non-porous, flat bottomed, approximate volume 50 mL)

10.2.5 Hamilton Microlab 500 Series Diluter

10.3 TESTING MEDIUM

10.3.1 Deionized water

10.4 ENVIRONMENTAL CONDITIONS

All equipment and testing media should be equilibrated to the ambient conditions present in the location where the procedure is performed. Electrical equipment should be turned on and allowed to stabilize for a minimum of one hour before use. Minimize air currents and temperature changes as much as possible.

The balance is to be placed on a marble work surface or comparable surface to minimize vibration. Handle weighing vessels with forceps or tweezers, not by hand.

Record the temperature, relative humidity and the serial number of the thermometer/ hygrometer. The temperature must be 22.0 ± 2 °C and the relative humidity between 40% and 75%.

10.5 SETUP

10.5.1 Keep the distance between the balance and diluter to a minimum.

10.5.2 Immerse the thermometer into the container holding the deionized water.

10.5.3 When sampling and dispensing, keep motions and time intervals as consistent as possible.

10.5.4 Minimize the weighing time cycles but don't compromise the integrity of liquid delivery.

10.6 PROCEDURE

1. Check the calibration of the balance by measuring reference weights and verifying that they are reading within the tolerances listed below. Record the balance model and serial number and the weight set model and serial number.
    - 10000 mg (± 0.050 mg)
    - 1000 mg (± 0.034 mg)

2. Using deionized water for both the reagent/diluent syringe (left position, 2000 µL) and for the sample syringe (right position, 200 µL), remove any air bubbles from the fluid path by slowly aspirating and then rapidly dispensing water several times.

3. Record the water temperature to 0.1 °C. Record the thermometer serial number and model number where applicable.

4. Open the door of the balance chamber, center the weighing vessel on the balance pan and close the door.

5. Tare the balance.

6. Open the door to the balance chamber, aspirate water and then dispense into the weighing vessel. Close the door and record the weight in milligrams, truncating to one decimal place.

7. Repeat steps 5 & 6 until you have recorded 10 total measurements.

10.7 CALCULATIONS

10.7.1 Open a Diluter Certification form and input the data recorded above.

10.7.2 Using the following table for density values, enter the density of water at the measured temperature into the Diluter Certification form.

Density of water (mg/μL) vs. Temperature (℃)

	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
20	0.998203	0.998183	0.998162	0.998141	0.99812	0.998099	0.998078	0.998056	0.998035	0.998013
21	0.997992	0.99797	0.997948	0.997926	0.997904	0.997882	0.99786	0.997837	0.997815	0.997792
22	0.997770	0.997747	0.997724	0.997701	0.997678	0.997655	0.997632	0.997608	0.997585	0.997561
23	0.997538	0.997514	0.99749	0.997466	0.997442	0.997418	0.997394	0.997369	0.997345	0.99732
24	0.997296	0.997271	0.997246	0.997221	0.997196	0.997171	0.997146	0.99712	0.997095	0.997069
25	0.997044	0.997018	0.996992	0.996967	0.996941	0.996914	0.996888	0.996862	0.996836	0.996809

Taken from CRC Handbook of Chemistry and Physics, 53rd edition, page F-4

10.7.3 Calculate the average volume using the following equation:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

10.7.4 Calculate the standard deviation using the following equation:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}}$$

10.7.5 Calculate the percent accuracy using the following equation (where R is the dispense volume in microliters):

$$\%\ accuracy = \left[\frac{\bar{X} - R}{R}\right] \times 100$$

10.7.6 Calculate the percent coefficient of variation using the following equation:

$$\%\ CV = \frac{SD}{\bar{X}} \times 100$$

10.8 ACCEPTANCE PARAMETERS

The diluter is to be considered certified for use if the gravimetric procedure produces the following results:

10.8.1 Average volume accuracy ± 3.0%

10.8.2 CV% ≤ 1.5%

If the procedure does not produce acceptable accuracy and precision then the diluter should be inspected for air in the fluid path, incorrectly-sized tubing, worn parts,

leaks or improperly tightened connections. Repairs should be documented and the gravimetric procedure repeated.

If in-house repairs do not resolve accuracy / precision results, then the diluter will be taken out of service and transferred to a manufacturer-supported service provider for service and calibration.

10.9 DOCUMENTATION AND REVIEW

Records of the certification will be made on a Diluter Certification form. The Diluter Certification will be provided to the QA Manager or designee for technical and administrative review.

The technical review will involve verification of calculations. The administrative review will verify the acceptable calibration status of equipment, verification of serial numbers and examination of the record for completeness. Upon completion of review, the diluter will be considered acceptable for use for a 12 month period from the date of the reviewer's signature.

11 PROCEDURE FOR THE GRAVIMETRIC CALIBRATION OF ADJUSTABLE, AIR DISPLACEMENT PIPETTES

11.1 POLICY

Each adjustable, air displacement pipette will be calibrated on an annual basis. If the pipette undergoes a manufacturer-provided repair which includes calibration or if calibration is contracted by the manufacturer or similarly approved service provider, then that calibration will satisfy the requirement for that year. Any outside calibration will be by an approved service provider that can show traceability to SI units and that will provide a calibration certificate (however named). If calibration is performed in-house, the following procedure must be followed. Performance verification is not covered in this procedure. Repeater-type pipettes only require annual performance verification.

Any adjustments or deviations from this procedure must be approved by the State Toxicologist or the QA Manager, and appropriately documented within the calibration record.

11.2 EQUIPMENT

11.2.1 Adjustable, air-displacement pipettes

11.2.2 Calibrated balance: Mettler XP205, or equivalent

11.2.3 Calibrated thermometer (0.1°C resolution or better)

11.2.4 Calibrated thermometer/hygrometer (for environmental monitoring)

11.2.5 Disposable pipette tips

11.2.6 Forceps or tweezers

11.2.7 Gloves (cloth or nitrile)

11.2.8 Reservoir for testing medium with cover to reduce evaporative cooling

11.2.9 Weighing vessels (variable-size, non-porous, flat bottomed, with lids for low volumes)

11.3 TESTING MEDIUM

11.3.1 Deionized water

11.4 ENVIRONMENTAL CONDITIONS

All equipment and testing media should be equilibrated to the ambient conditions present in the location where the procedure is performed for at least 2 hours prior to use. Minimize air currents and temperature changes as much as possible.

The balance is to be placed on a marble work surface or comparable surface to minimize vibration. It should be located away from any windows or doors. Minimize

conductive heating by wearing gloves during the procedure and use forceps or tweezers to handle weighing vessels used for volumes < 100µL.

Record the temperature, relative humidity and the serial number of the thermometer/ hygrometer. The temperature must be between 15°C and 30°C and the relative humidity between 40% and 65%. The temperature should not vary by more than ±1.0°C during calibration.

11.5 BALANCE

A 5-place balance is used for measurement. A 5-place balance is capable of displaying 10^{-5} grams or 0.01 mg. Table 1 describes balance sensitivity required for pipettes with the stated nominal (maximum) volumes. In the absence of a balance with sufficient sensitivity, low nominal volume pipettes will be calibrated annually by an approved service provider.

Table 1

Sensitivity (g)	Display	Nominal pipette volume in µL
10^{-6}	0.000 mg	20, 25
10^{-5}	0.00 mg	100, 200, 250
10^{-4}	0.0 mg	1000, 2000, 2500, 5000, 10mL

The balance will have been certified for use within the last 12 months and this will be verified by examining the certificate of calibration (however named) of the service provider. Balance certification will have utilized reference standards traceable to SI units.

11.6 SETUP

11.6.1 Keep the distance between the balance and pipette to a minimum.

11.6.2 Immerse the thermometer into the deionized water reservoir. Cover the reservoir when not sampling.

11.6.3 When sampling and dispensing, keep motions and time intervals as consistent as possible.

11.6.4 Endeavor to keep the weighing time cycles under 60 seconds.

11.6.5 The pipettes should be cleaned and any maintenance service (ex. lubrication) conducted prior to taking gravimetric measurements.

11.7 PIPETTE OPERATION

Use the following guidelines when operating the pipette.

1. Change tips whenever volumes are changed.

2. Pre-rinse tips before performing any sampling.

3. Dispense the sample by touching the pipette tip to the side wall of the weighing vessel.

4. Operate the pipette in the vertical position.

5. For sampling, use the following table as a guide for tip immersion.

Table 2	
Immersion Depth (mm)	Volume to be sampled (µL)
2-3	1 to 100
2-4	100 to 1000
2-5	1000 to 5000

6. Use the forward pipetting technique: Depress the plunger to the first stop, placing the tip into the sample to the proper immersion depth, slowly release the plunger to the up position, pause briefly to ensure the full volume has been sampled, touch the pipette tip to the side of the sample reservoir to remove any drops, touch the tip against the side wall of the weighing vessel just above the liquid surface at a 30-45° angle, slowly depress the plunger to the first stop, pause for 1-2 seconds, press the plunger to the second stop, draw the tip 8-10mm up the side of the weighing vessel and withdraw the pipette.

11.8 EVAPORATION RATE ESTIMATION

The effect of evaporation on the measurement must be factored when certifying pipettes with nominal values less than 100 µL. This is done by performing a set of simulated weighings, calculating the mean evaporation rate and factoring that rate into the calculation of volumes.

1. Using one of the small weighing vessels with accompanying lid, place water into the weighing vessel to 1/3 full.

2. With the lid on, place the weighing vessel on the balance.

3. Sample a portion of water using a pipette.

4. Tare the balance and then remove the weighing vessel.

5. Remove the lid from the weighing vessel.

6. Dispense the water from the pipette back into the water reservoir, NOT into the weighing vessel.

7. Replace the lid and return the vessel to the balance.

8. Record the weight (e_i), even if negative.

9. Repeat steps 3 through 8 nine more times.

10. Calculate the average evaporation loss in mg using the following equation:

$$\bar{e} = (e_1 + e_2 + e_3 + \cdots e_{10}) \div 10$$

11. Convert to a positive value with 2 decimal places.

11.9 PROCEDURE *(3 Volumes X 10 Weighings, Addition – Tare Method)*

1. Verify that environmental conditions are within specifications and record the values.

2. Fill the weighing vessel to at least 3mm depth with water.

3. The 3 volumes selected will correspond to:
   a. The nominal volume (100%)
   b. 50% of nominal
   c. 10% of nominal or the minimum recommended volume whichever is greater.
   d. Examples: a 100µL pipette has 10 weighings at 10, 50 and 100µL; a 200µL pipette with a 50µL minimum recommended has 10 weighings at 50, 100 and 200µL.

4. Set the pipette volume by adjusting one-third turn past the test volume and then adjusting down to the test volume.

5. Record the water temperature to the nearest 0.5°C. Record the thermometer serial number and model number when applicable.

6. Open the door of the balance chamber, center the weighing vessel on the balance pan and close the door.

7. Tare the balance.

8. Fill the pipette tip with water and expel to waste five times to reach humidity equilibrium in the dead air volume of the pipette.

9. Replace the pipette tip and pre-rinse once.

10. Aspirate a water sample.

11. Open the door to the balance chamber, remove the weighing vessel and then dispense the test volume into the weighing vessel. (If adequate space allows, the weighing vessel may remain on the balance during sample delivery)

12. Return the weighing vessel to the balance and close the door.

13. Record the weight in milligrams to two decimal places.

14. Repeat steps 7 through 13 until you have recorded 10 total measurements.

15. Record the water temperature to the nearest 0.5°C. The average water temperature rounded to the nearest 0.5°C is used to designate the Z-factor (see calculations below).

16. Repeat the process for the remaining two volumes, verifying the environmental conditions and recording the water temperature before and after each set of measurements.

11.10 CALCULATIONS

11.10.1 Convert individual measurement masses to volumes by multiplying by the Z-factor for the average water temperature (see Table 3 below).

$V_i = Z \times m_i$

- V_i = individual volumes (µL)
- Z = Z-factor (µL/mg)
- m_i = individual mass (mg)

Table 3

Z-factor (101.3 kPa)			
Temp(°C)	Z-Factor (µL/mg)	Temp(°C)	Z-Factor (µL/mg)
15.0	1.0020	20.0	1.0029
15.5	1.0020	20.5	1.0030
16.0	1.0021	21.0	1.0031
16.5	1.0022	21.5	1.0032
17.0	1.0023	22.0	1.0033
17.5	1.0024	22.5	1.0034
18.0	1.0025	23.0	1.0035
18.5	1.0026	23.5	1.0036
19.0	1.0027	24.0	1.0038
19.5	1.0028	24.5	1.0039

o For pipettes with nominal values less than 100µL, the individual weights are corrected for evaporation by first adding the average evaporation rate to the weight before multiplying by the Z-factor.
$V_i = Z(m_i + \bar{e})$

11.10.2 Calculate the mean volume $(\overline{V})$ using the following equation:

$$\bar{V} = \frac{1}{n}\sum_{i=1}^{n} V_i$$

11.10.3 Calculate the precision as standard deviation (random error) using the following equation:

$$s_r = \sqrt{\frac{1}{n-1}\left(\sum_{i=1}^{n}(V_i - \bar{V})^2\right)}$$

11.10.4 Calculate the systematic error (inaccuracy) as a percent using the following equation:

$$e_s = 100 \times (\bar{V} - V_s)/V_s$$

- V_s = test volume

11.10.5 Calculate the random error (imprecision) as percent coefficient of variation using the following equation:

$$CV = 100 \times s_r/\bar{V}$$

11.10.6 Calculate the expanded uncertainty (k=2, 95% confidence interval) using the following equation:

$$u = |e_s| + 2s_r$$

11.11 ACCEPTANCE PARAMETERS

Pipettes are to be considered certified for use for one year if they meet the specifications in Table 4. If the calibration procedure does not produce acceptable accuracy and precision then the pipette can be adjusted following manufacturer guidelines and the certification procedure repeated. Any adjustment will be recorded on the Pipette Calibration form.

If in-house adjustment does not resolve accuracy/precision results, then the pipette will be taken out of service and transferred to an approved calibration service provider.

Table 4

Nominal Volume (µL)	Test Volume (µL)	Inaccuracy		Imprecision	
		µL (±)	%	µL (≤)	%
20	2	0.06	3.0	0.04	2.0
	10	0.12	1.2	0.1	1.0
	20	0.18	0.9	0.08	0.4
100	10	0.2	2.0	0.1	1.0
	50	1.0	2.0	0.5	1.0
	100	2.0	2.0	1.0	1.0
200	20	0.4	2.0	0.2	1.0
	100	2.0	2.0	1.0	1.0
	200	4.0	2.0	2.0	1.0
1000	100	2.0	2.0	1.0	1.0
	500	10.0	2.0	5.0	1.0
	1000	20.0	2.0	10.0	1.0
5000	500	10.0	2.0	5.0	1.0
	2500	50.0	2.0	25.0	1.0
	5000	100.0	2.0	50.0	1.0

11.12 DOCUMENTATION AND REVIEW

Records of the calibration will be made on a Pipette Calibration form. The Pipette Calibration record will be provided to the QA Manager or designee for technical and administrative review. Record any cleaning, preventative maintenance, repair or adjustment made on the Pipette Calibration form.

The technical review will involve verification of calculations. The administrative review will verify the acceptable calibration status of equipment, verification of serial numbers and examination of the record for completeness. Upon completion of review, the pipette will be considered acceptable for use for a 12 month period from the date of the reviewer's signature.

Pipette calibration records will be maintained in the equipment log for each pipette, which will be maintained by the QA Manager or designee.

12 PROCEDURE FOR GRAVIMETRIC PERFORMANCE VERIFICATION OF ADJUSTABLE, AIR DISPLACEMENT PIPETTES

12.1 POLICY

Each adjustable, air displacement pipette purchased by the Laboratory will have its performance verified by this procedure prior to being placed into service. This procedure will also be used for intermediate performance verification of calibrated pipettes when adequate performance is suspect, and will be used for the annual performance verification of repeater-type pipettes. Pipette calibration is not covered in this procedure.

Any adjustments or deviations from this procedure must be approved by the State Toxicologist or the QA Manager, and appropriately documented within the verification record.

12.2 EQUIPMENT, TESTING MEDIUM, ENVIRONMENTAL CONDITIONS

12.2.1 See "Procedure for the Gravimetric Calibration of Adjustable, Air Displacement Pipettes (11.0)"

12.3 BALANCE

A 5-place balance is used for measurement. A 5-place balance is capable of displaying 10^{-5} grams or 0.01 mg. Table 1 describes balance sensitivity required for pipettes with the stated nominal (maximum) volumes. For the purpose of performance verification, the 5-place balance may be used for pipettes with nominal volumes below 100µL.

Table 1

Sensitivity (g)	Display	Nominal pipette volume in µL
10^{-6}	0.000 mg	20, 25
10^{-5}	0.00 mg	100, 200, 250
10^{-4}	0.0 mg	1000, 2000, 2500, 5000, 10mL

The balance will have been certified for use within the last 12 months and this will be verified by examining the certificate of calibration (however named) of the service provider. Balance certification will have utilized reference standards traceable to SI units.

12.4 SETUP, PIPETTE, OPERATION

12.4.1 See “Procedure for the Gravimetric Calibration of Adjustable, Air Displacement Pipettes (11.0)”

12.5 EVAPORATION RATE ESTIMATION

12.5.1 See “Procedure for the Gravimetric Calibration of Adjustable, Air Displacement Pipettes (11.0)”

12.6 PROCEDURE *(2 Volumes X 4 Weighings, Addition – Tare Method)*

1. Verify that environmental conditions are within specifications and record the values.

2. Fill the weighing vessel to at least 3mm depth with water.

3. The 2 volumes selected will correspond to:
   a. The nominal volume (100%)
   b. 25% of nominal
   c. Repeater-pipettes will use the maximum and 10% settings. The test volumes will depend on the tip size, and they will be noted on the pipette verification record.

4. Set the pipette volume by adjusting one third turn past the test volume and then adjusting down to the test volume. Set repeater-type as normal.

5. Record the water temperature to the nearest 0.5°C. Record the thermometer serial number and model number where applicable.

6. Open the door of the balance chamber, center the weighing vessel on the balance pan and close the door.

7. Tare the balance.

8. Fill the pipette tip with water and expel to waste five times to reach humidity equilibrium in the dead air volume of the pipette. Not applicable for repeater-type.

9. Replace the pipette tip and pre-rinse once. Not applicable for repeater-type.

10. Aspirate a water sample.

11. Open the door to the balance chamber, remove the weighing vessel and then dispense the test volume into the weighing vessel. (If adequate space allows, the weighing vessel may remain on the balance during sample delivery)

12. Return the weighing vessel to the balance and close the door.

13. Record the weight in milligrams to two decimal places.

14. Repeat steps 7 through 13 until you have recorded 4 total measurements.

15. Record the water temperature to the nearest 0.5°C. The average water temperature rounded to the nearest 0.5°C is used to designate the Z-factor (see calculations below).

16. Repeat the process for the second volume, verifying the environmental conditions and recording the water temperature before and after each set of measurements.

12.7 CALCULATIONS

12.7.1 See "Procedure for the Gravimetric Calibration of Adjustable, Air Displacement Pipettes (11.0)". Measurement uncertainty is not calculated for performance verifications.

12.8 ACCEPTANCE PARAMETERS

Pipettes are to be considered verified if they meet the specifications in Table 2. Repeater-type pipette specifications are ± 2.0% systematic error and ± 1.0% random error for all volumes. If the performance verification procedure does not produce acceptable accuracy and precision then the pipette can be adjusted following manufacturer guidelines. Following any adjustment, the pipette must be calibrated.

If in-house adjustment and calibration does not resolve accuracy/precision results, then the pipette will be taken out of service and transferred to an approved calibration service provider or returned to the manufacturer.

Table 2

Nominal Volume (µL)	Test volume (µL)	Inaccuracy		Imprecision	
		µL (±)	%	µL (≤)	%
20	5	0.15	3.0	0.1	2.0
	20	0.4	2.0	0.2	1.0
100	25	0.5	2.0	0.25	1.0
	100	2.0	2.0	1.0	1.0
200	50	1.0	2.0	0.5	1.0
	200	4.0	2.0	2.0	1.0
1000	250	5.0	2.0	2.5	1.0
	1000	20.0	2.0	10.0	1.0
5000	1250	25.0	2.0	12.5	1.0
	5000	100.0	2.0	50.0	1.0

12.9 DOCUMENTATION AND REVIEW

Records of the calibration will be made on a Pipette Verification form. The Pipette Verification record will be provided to the QA Manager or designee for technical and administrative review. Record any cleaning, preventative maintenance or repair on the Pipette Verification form.

The technical review will involve verification of calculations. The administrative review will verify the acceptable calibration status of equipment, verification of serial

numbers and examination of the record for completeness. Upon completion of review, new pipettes will be considered acceptable for use for a 12 month period from the date of the reviewer's signature. For intermediate performance verifications, the pipette will be considered acceptable for use until its next calibration due date. Repeater-type pipettes will be considered acceptable for use for a 12 month period from the date of the reviewer's signature.

Pipette verification records will be maintained in the equipment log for each pipette, which will be maintained by the QA Manager or designee.

13 RECORDS, REVIEWS AND REPORTS

13.1 POLICY

All records created by the TLD will be identifiable, accessible to authorized personnel and properly stored to prevent damage or loss. Electronic documentation will be backed-up and should be protected to prevent unauthorized access to or amendment of these records. Records will include the identity of personnel responsible for the performance of each function, and the reviewing and issuing of results.

13.2 DEFINITIONS

13.2.1 Administrative Documentation: Documentation either received or generated by the Laboratory. Administrative documentation includes records such as reagent receipts, certificate of analyses, and other pertinent information.

13.2.2 Technical Documentation: Usually generated by the Laboratory and includes reference to standard operating procedures (SOP's) used in testing, identity of tests conducted, standard and control traceability, instrument data reports, results of tests, technical reviews, etc.

13.2.3 Batch File: A batch file contains both administrative and technical documentation pertaining to a particular test conducted by an analyst in the Laboratory. This may include, but is not limited to:

- Sequence table or worklist
- Chromatograms and/or data reports
- Traceability information (for standards, positive controls, blank matrices)
- Documentation of technical review

13.2.4 Batch Record: A batch record is a collection of all the administrative and technical documentation pertaining to a particular test conducted by an analyst in the Laboratory. This may include, but is not limited to:

- Electronically stored data
- Instrument maintenance and/or verification documentation
- Reagent and standard quality control documentation

Information in the batch record may be in the batch file or in other locations in the Laboratory which are designated as extensions of the batch file.

13.2.5 Toxicology Report: Final presentation of results of testing conducted in the Laboratory.

13.3 TESTING DOCUMENTATION

13.3.1 Administrative Documentation

Administrative documentation should bear some unique identifier in order to be placed back into its source file if it becomes separated. If the administrative documentation is a packet of material that is fastened together, the unique identifier need only be on the first page.

13.3.2 Technical Documentation

Technical documentation should bear some unique identifier. For a batch file, this is the batch name, relating the documentation to the instrument, analyst, type of testing and date the testing was performed. For a case file, this is the Laboratory's assigned ST#.

13.3.3 General Documentation Requirements

Handwritten documentation will be recorded using permanent ink.

Nothing in the testing documentation may be erased or obliterated. Changes, additions, or any other form of alteration must be initialed and dated by the person making the alteration. Overwrites should be struck-through, rewritten, and initialed/dated.

For records that are duplicated in electronic format, such as for public disclosure or legal discovery purposes, corrected originals will be copied to, but will not replace, the electronic duplicates. Amended reports will be duplicated in electronic format and will be added to the electronically duplicated records of the original report.

Dates must be recorded in the documentation to indicate when work was performed.

Abbreviations are acceptable if they are readily comprehensible to a reviewer or if a key is available.

Testing documentation includes but is not limited to the following:

- Results of testing (e.g. chromatograms and data reports)
- Records of data and calculations
- Handwritten or machine-generated worksheets
- Identity and source of any standards or references used

When instrumentation is used, the specific instrument used must be noted in the batch file. If the Laboratory has only one instrument for a specific test or procedure, that instrument's identification may be documented in the Laboratory's equipment list. If the

Laboratory has multiple instruments of the same make/model, the unique identifier of the instrument used must be recorded in the batch file.

Observations, data and calculations must be recorded at the time they are made, and must be identified to the specific analysis or test.

Documentation to support the results shall be such that in the absence of the analyst, another competent analyst or Supervisor could evaluate what was done and interpret the data.

Documentation of the technical peer review is discussed below.

13.4 REVIEW OF RECORDS

13.4.1 Policy

The Laboratory will ensure that reports are accurate and supported by the technical documentation, and that established policies and procedures are being followed. All laboratory reports and associated documentation will be subject to technical and administrative reviews.

13.4.2 Definitions

13.4.2.1 Administrative Review: Final review for non-technical matters of the case file and final report prior to release of the report to the customer.

13.4.2.2 Supervisor Review: A general review of testing records by a supervisor to maintain oversight of laboratory operations.

13.4.2.3 Technical Review: A review of the testing documents to ensure that proper technical procedures were used and documented and any applicable acceptance criteria have been met.

13.4.3 Procedure

Review of testing information by the analyst, and other personnel, provides a verification of procedures and results.

13.4.3.1 Analyst Review

Analysts will conduct a thorough review of their own work, including the review of batch files and case files prior to submission to authorized personnel for technical an administrative review.

13.4.3.2 Technical Review

Technical review will be conducted on all testing data to ensure that the appropriate test procedures were followed and that any necessary criteria have been met.

The technical review should include, but is not limited to, the following:

- The appropriate test procedures were followed
- The appropriate test data and any supporting documentation is included
- Criteria for acceptance described in the relevant test procedure has been met
- Any deviations from established procedures were recorded in the record with adequate justification/foundation for the deviation
- Standards and controls used were appropriate and traceability information documented
- All strikeouts or insertions were noted with the analyst's initials and date. No obliterations are present

Technical review will be documented with the reviewer's signature/initials and date in the batch file.

13.4.3.3 Administrative Review

An administrative review will be conducted on the case file prior to the release of written reports, including amended reports. Administrative case review is detailed in section 5.0 of the Administrative Procedures.

13.5 FOCUSED REVIEW

When internal quality processes uncover serious errors in testing work, or there is a complaint alleging misconduct or incompetence, the Appointing Authority may initiate a focused testing work review. If a root cause analysis has been completed, the Appointing Authority will review the analysis and its recommendations and any other input from the QA Manager as part of their deliberation as to the necessity of a focused casework review.

13.5.1 Review of Affected Testing Work

The focused testing work review will be conducted by an appropriate Supervisor or panel chosen by the Appointing Authority. The reviewing Supervisor will prepare a report summarizing the findings and forward the report to the QA Manager who will review and discuss the report with the Appointing Authority.

13.5.2 Notifications

The notification must be made as soon as practical but not later than 30 days after the review begins. The notification will include the fact that a review or audit is being conducted and will identify all testing work under review.

If the Toxicology Report reflecting results obtained from the testing work in question has been released prior to the commencement of the focused testing work review, the Appointing Authority will notify the customer as soon as practical but not later than 30 days after the review begins.

13.5.3 Removal from and Reinstatement to Testing Work

The analyst who is under review will be removed from testing work by the Appointing Authority until the matter is resolved. In addition to the fact finding, technical review, re-examination of work, or other action taken by Laboratory Management, an amended Toxicology Report may be issued to the customer, with copies sent to the prosecuting attorney's office, where necessary. Reinstatement to testing work will also be by the Appointing Authority.

14 TRACEABILITY AND QUALITY CONTROL

Many factors contribute to the accuracy and reliability of testing performed by the TLD, including:

- The training and qualifications of personnel
- Technical/analytical methods
- Reagents and supplies
- Selection, verification and maintenance of equipment

The TLD will take into account these and other factors and will ensure that the personnel are properly qualified and trained; that procedures are validated; that reagents and supplies are traceable and/or verified for performance; and that equipment is calibrated and/or verified. All procedures, reagents, supplies and equipment/instrumentation will be controlled.

14.1 TRACEABILITY AND QUALITY CONTROL OF REAGENTS

14.1.1 Policy

All commercially and laboratory prepared reagents, as well as chemicals used to prepare reagents, will be of sufficient quality to assure the integrity of the results. Reagents prepared in the laboratory should be labeled with the identity of the reagent, the preparer's initials, the date of preparation, and the expiration date. Records of reagent preparation shall be maintained, including that its reliability was verified prior to use, where applicable.

Section 4.1 details the procedure for receipt and verification of reagents and maintenance of related records.

14.2 VALIDATION OF EQUIPMENT AND INSTRUMENTATION

14.2.1 Policy

Instrumentation to be used must be validated prior to being placed in service in the TLD. Instrumentation to be used for existing applications and methods must have performance verified before initial use. The purpose is to establish that it is capable of achieving the Laboratory's and the manufacturer's specifications for the test.

All instruments and major equipment will be uniquely identified. Equipment/instruments will have regular maintenance and performance verifications to ensure continued performance. Maintenance, calibration and verification procedures will be documented and maintained in an equipment/instrument maintenance and/or verification logbook. In addition, equipment/instruments will only be operated by authorized personnel. Each section will maintain a list of persons authorized to operate the equipment/instrumentation.

14.2.2 Definitions

14.2.2.1 Calibration: The process by which standards having known reference values are introduced into an instrument. The instrument is then adjusted or programmed (either by software, hardware, electronics, etc.) to report the known reference value.

14.2.2.2 Performance Verification: Performance verification is a set of operations to determine if a piece of equipment or instrumentation is working correctly within manufacturer's specifications or TLD's specified parameters.

14.2.2.3 Traceability: The property of a measurement result whereby it can be related to standard references, usually national or international, through an unbroken chain of comparisons.

14.2.3 Procedure

All analytical equipment/instruments and any associated software will have records that are maintained in an equipment/instrument maintenance and/or verification logbook. This logbook will be kept in the laboratory, and in close proximity to the equipment/instrument, whenever possible. The laboratory will maintain retired logbooks for at least one accreditation cycle. An electronic logbook is an acceptable alternative to a written log.

Where applicable, the following information should be kept in the Equipment/Instrument logbook:

- The equipment/instrument identity: type, manufacturer, model, serial number or unique name and current location
- The original equipment paperwork provided with instrument installation, wherever possible
- The maintenance plan and/or procedure and records of maintenance performed
- Date of maintenance, initials of the person doing the maintenance and activity conducted
- Performance verification procedures
- Documentation of performance verification
- Scheduled calibration (if required) including dates, results, reports and certificates
- Any damage, malfunction, modification or repair to the equipment/instrumentation

Each instrument will be uniquely identified and the identifier will be used in all documentation, including any reports or hard copy instrument data.

Where applicable, other equipment/instrument documentation to be maintained includes:

- The Manufacturer's maintenance and operating manuals or reference to their location
- Internal validation procedure, data and documentation

14.2.4 Equipment/Instrument Maintenance

Maintenance procedures will include a maintenance plan that indicates the frequency and type of maintenance to be performed (i.e., annual, as needed, by manufacturer, etc.) and any scheduled manufacturer maintenance contract information (if applicable).

For equipment, calibration check intervals will not be less stringent than that recommended by the manufacturer. The maintenance plan will be located in the technical manuals and/or the maintenance logbook.

14.2.5 Equipment/Instrument Performance Verification

The Laboratory will ensure that all equipment/instrumentation, either newly purchased or existing, are properly validated or have their performance verified prior to use. The process will be as extensive as is necessary to meet the needs of the given application. All validation/verification studies will be performed by qualified personnel with adequate resources to perform the study.

Performance verification procedures will be documented in the equipment/instrument and/or verification logbook. Verification procedures will include verification requirements (e.g. frequency of verification and tolerances, acceptance criteria) and specific step-by-step verification protocols, including the use of any reference standards. When possible, all verification will be completed with traceable reference standards or materials.

The minimum information that will be recorded in the equipment/instrument and/or verification logbook will include the following:

- The instrument unique identifier or name, model and serial number
- The verification date
- Initials of the person performing the verification
- The type of verification performed (post-maintenance, scheduled, etc.)
- If the instrument passed or failed performance verification
- Identification of reference material used, where applicable
- Any comments regarding the performance check

Equipment/instrumentation that does not meet performance specifications shall be taken out of service. The instrument will be clearly labeled or marked as being “Out of Service” until it has been repaired or evaluated, and shown by calibration or performance verification to perform within specifications. In addition, the removal of the instrument from service should be documented in the equipment/instrument log and should indicate why the instrument was removed from service. The date the instrument is placed back in service should also be indicated in these logs.

If the nature of the malfunction is such that the accuracy of previous reported test results are suspect, the situation shall be immediately brought to the attention of the Supervisor and the QA Manager. The QA Manager will inform the TLD Commander, and corrective action shall be performed.

14.2.6 Equipment Calibration

Analytical equipment requiring calibration (e.g. diluters, analytical balances, pipettes) will be calibrated prior to being implemented in the laboratory.

Calibration status will be checked after any unexpected shutdown or removal of the equipment from service and following service or other substantial maintenance.

Equipment calibration will be described in the technical manuals and/or maintenance logbook.

Equipment requiring calibration will have a documented calibration schedule, including the frequency of calibration required, the status of calibration and the next calibration due date. Calibration/recalibration documentation and calibration certifications will be maintained on file at the laboratory.

Whenever practicable, all equipment requiring calibration will be labeled or identified to indicate the status of calibration. This should include the date when last calibrated and the date when recalibration is due.

When external calibration services are used, traceability of measurement will be assured by the use of services that can demonstrate competence, measurement capability and traceability. The calibration certificates issued by these services will contain the measurement results, including the measurement uncertainty and/or a statement of compliance with an identified metrological specification.

14.2.7 Responsibilities:

Forensic Scientists are responsible for:

- Performing assigned instrument verification and maintenance and documenting all necessary information concerning verification and maintenance activities in the instrument logbooks
- Ensuring that the equipment in use has been properly calibrated or verified prior to use

Laboratory Managers/Supervisors are responsible for:

- Ensuring that calibration/verification and maintenance procedures are in place for each instrument determined to require verification and maintenance
- Monitoring compliance with calibration/verification and maintenance procedures through periodic spot checks

- Addressing problems concerning verification according to TLD Policy
- Ensuring that only approved external calibration providers used by the Laboratory
- Ensuring that all users are authorized prior to instrument use
- Ensuring that required calibration/verification and maintenance as outlined in the written procedures are carried out, and according to schedule
- Periodic review of all calibration/verification and maintenance records and activities

The QA Manager is responsible for:

- Monitoring compliance with calibration/verification and maintenance procedures
- Conducting an annual review and update of this policy

The TLD Commander is responsible for:

- Monitoring all instrument calibration/verification and maintenance activities through review of annual audit reports and other communications through laboratory employees

14.3 TRACEABILITY OF MEASUREMENT STANDARDS

14.3.1 Policy

All equipment/instrumentation used in the laboratory, having a significant effect on the measurement result and their associated uncertainties of measurement, will be traceable to national and/or international standards of measurement. This will be done through the use of a measurement standard. The TLD will safely handle, transport and store these measurement standards in order to prevent contamination or deterioration and in order to protect their integrity.

14.3.2 Definitions

14.3.2.1 National/International Standard

A standard recognized by national or international agreement to serve as the basis for assigning values to other standards of the quantity concerned. The standards which generally apply are the metric system of measures expressed in SI units, the units of the International System of Units.

14.3.2.2 National Institute of Standards and technology (NIST)

This federal agency, also known as NIST, is located within the U.S. Department of Commerce and represents the final authority for metrology in the United States. Ideally, all measurement results should be documented and shown to be traceable to NIST.

14.3.2.3 Reference Material Producer

An organization or firm which manufactures and provides certified reference materials for the purpose of ensuring traceability and estimated uncertainty. The producer shall be responsible for assigning a reference value to the material along with any available uncertainty.

14.3.2.4 Certified Reference Material (CRM)

A material or substance, accompanied by a certificate, one or more of whose property values are certified by a procedure that establishes traceability to an accurate realization of the unit in which the property values are expressed. Each certified value is accompanied by an uncertainty. An example of such a CRM would be a NIST traceable thermometer.

14.3.3 Procedure

Reference standards or materials (e.g. weights) used to check accuracy of other equipment or instruments shall not be used for other purposes.

Adjustments and/or calibration of reference materials shall only be conducted by approved, external calibration service providers. All calibrations and adjustments to these materials will be documented.

Wherever possible, vendors used for calibration or recertification of these standards shall be certified or accredited by ISO or other international/national accrediting bodies.

Following service, maintenance and recalibration by such vendors, the certification or documentation provided by them will be maintained in the laboratory.

If mishandling of standards brings accuracy into question, the standards shall be taken out of service and recalibrated.

When traceability of measurements cannot be made in or is not relevant to SI units, then reference materials will establish traceability by one of the following:

- The use of certified reference material from a supplier
- The use of specified methods, published standards
- Participation in inter-laboratory comparisons

Documentation of this traceability to SI units or CRMs and the recalibration/recertification information shall be maintained at the laboratory.

15 LIST OF CHANGES

Revision Date	Procedure	Change	Page Number
07/08/13	Original	Document approved by the State Toxicologist. See DRA dated 07/08/13. Effective date 07/08/13.	All