North Carolina Department of Health and Human Services
Office of the Chief Medical Examiner

Quality Assurance and Quality Control Manual

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North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	Revision:	Revision Date/Initials:
	2.6.4, 2.6.5 – Cal curve and CCAL acceptance criteria updated 2.9.10.1 – QC acceptance criteria updated	MSF 04/30/2015 MSF 04/30/2015
	2.13.4, 2.13.4.1 – Ion ratio acceptance criteria/update criteria/exceptions updated 2.12.1.1 – Updated RT acceptance criteria	MSF 04/30/2015
	2.8.2 – Updated Calibrator acceptance range	MSF 09/22015
	1.4.3.8.2 – Added controlled substance reporting exception	MSF 04/04/2016
	1.7.3.7 – Added 1.8.2 – Updated Special Analysis NTF 1.8.3 – Deleted – Changes to sample size 2.6.6 - Added Assay pending validation protocol 2.7 – Updated (Quant) 2.9.12.3 – Updated blank reinjection protocol 2.9.12.3.3 – Updated contaminated blank procedure 2.17.7 – updated reporting drug conc. in urine 2.19.3.1 – updated language 5.3 – updated language 7.1.7 & 7.1.9 – updated reagent logging and labeling 7.3.1 – deleted addition of salt (blank liver hd) 7.3.3 – updated blank liver storage (-20C)	MSF 03/14/2017
	2.13.1 – Updated language 2.2.3-2.2.4 – Added standard prep guidelines 1.8.2 – Updated authorization for Special Analysis NTF 2.17.2.1 -Updated reporting average of 6 (truncated) 1.7.3.5.3 – Updated in-house PT acceptance ranges 2.15.1.6 – Updated carryover guidelines 4.1.7.2 – Added instructions for weighing specimens with serial dilutions. 4.1.9.4 – Updated procedure for reporting total gastric	MSF - 05/09/2017 MSF – 05/25/2017 MSF – 05/26/2017 MSF – 082417
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Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/14/2015
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1. Toxicology Quality Assurance Guidelines

1.1. Introduction

1.1.1. The purpose of this manual is to provide a uniform Quality Assurance Program for the Toxicology Laboratory of the Office of the Chief Medical Examiner. It is designed to ensure that the parameters of the testing process are routinely monitored in a manner that maintains the success and reliability of the analytical results. This manual should be used in conjunction with:

1.1.1.1. Toxicology SOP Manual
1.1.1.2. NC-OCME Policy and Procedures Manual
1.1.1.3. Safety Manual/Chemical Hygiene Plan
1.1.1.4. NC OCME Training Manual

1.1.2. Since most forensic postmortem toxicology specimens are limited in quantity or volume, it is highly desirable to minimize the need for repeat analysis due to the failure of equipment, materials or reagents. The focus of the quality assurance program is to identify potential problems before they become issues necessitating corrective action.

1.1.3. It is expected that the analyst will report any unacceptable or anomalous behavior of any analytical system immediately to either their supervisor or the appropriate senior chemist (in accordance with TOX-P4 Toxicology Employee Policy). It is further expected that appropriate actions will follow as soon as possible and be properly documented.

1.1.4. All employees are expected to behave in an honest, ethical, and professional manner (in accordance with TOX-P4 Toxicology Employee Policy).

1.1.5. The Quality Assurance program, as a whole, is to be reviewed annually by the QA/QC Chemist in conjunction with other senior chemists (Chemist II and above).

1.1.5.1. The review will include randomly selected case work, a follow up of corrective actions, as well as an assessment of the appropriateness of QC in individual assays.

1.1.5.2. The annual review will be approved by the laboratory director with a signature on the revision table found in this document.

1.2. Standard Operating Procedures (SOP), Laboratory Policies, and Document Control

1.2.1. All documents within the OCME Toxicology Laboratory are controlled so that any changes to an official document are recorded.

1.2.1.1. SOPs and Policies: A revision table is part of each SOP and Policy.

1.2.1.1.1. Any changes to an SOP or Policy are recorded in the revision table along with the date of the change and the initials of the person proposing the change.

1.2.1.1.2. An approving signature of the Chief Toxicologist is needed before the change is accepted and finalized.

1.2.1.1.3. A copy of the old SOP or Policy is saved in the "Archived SOP" or "Archived Policy" folder as appropriate with the date in service included in the file name (e.g. SOP 101_012110to030413).

1.2.1.1.4. A copy of the new SOP or Policy is saved in the current "SOP" or "Policy" folder as appropriate with the date in service included in the file name (e.g. SOP 101_030413).

1.2.1.1.5. All bench references affected by the change(s) will be replaced by an updated copy reflecting the change(s).

1.2.1.2. All non-electronic laboratory documents, such as Standard and Control worksheets and other batch analysis documentation, will be forensically corrected whereas the information to be changed is crossed out with a single horizontal line, the new information neatly written nearby, and the change(s) initialed and dated by the person making the change.

1.2.2. All laboratory SOPs and Policies are reviewed annually by the laboratory director or designee.

1.2.2.1. The initials and date of review will be recorded in the "Revision and Approval" section of each document.

1.3. Summary

1.3.1. The following toxicology quality guidelines apply for the analysis and subsequent accepting and reporting of toxicology results, unless otherwise specified in a specific method SOP.

1.3.2. Minor deviations and exceptions shall be authorized by a senior chemist or toxicologist and documented in the case file with a note-to-file (NTF).

1.3.3. Major deviations and exceptions shall be authorized by the Chief or Deputy Chief Toxicologist and documented in the case file with a NTF.

1.3.4. Whenever an incorrect result is certified and signed out by a reviewing toxicologist or senior staff member, a corrective action report (CAR) must be completed (see TOX-P20) Dissemination and Interpretation of Toxicological Results). The toxicology report will be re-issued and reflect both the corrected result as well as the previously reported incorrect result.

1.4. Guidelines for Confirming Positive Results

1.4.1. As a general matter of forensic principle, the detection of drugs and other toxins should be confirmed (whenever possible) by a second technique based on a different analytical principle.

1.4.2. If a second technique is not available, the identification should be confirmed on a different aliquot of the same specimen or, preferably, in an alternate specimen.

1.4.3. The following are acceptable confirmatory practices. At least one condition must be satisfied in order to identify and report a drug.

1.4.3.1. Identification of the substance and specific identification of the substance in a different aliquot by a different chemical principle (e.g. GC/MS full scan (screen) followed by GC/MS SIM or LC/MS/MS quantitation).

1.4.3.2. Identification of the substance in more than one aliquot by different chemical principles (e.g. organic base screen identification of antidepressant by GC/MS followed by quantitation by GC/NPD or color test for salicylate followed by quantitation by LCMS).

1.4.3.3. Identification of the substance in different biological samples by one or more chemical principles (e.g. positive LC/Ion Trap/MS screen for morphine in central blood, confirmation of morphine by LC/MS/MS in peripheral blood).

1.4.3.4. Identification of the substance in one biological sample using two separate aliquots and slightly different analytical principle (e.g. ethanol analysis by headspace GC/FID, BAC1 and BAC2 columns).

1.4.3.5. Identification of an incidental substance by full scan GC/MS in one aliquot and case history verifies the identification (e.g. lidocaine confirmed in organic base screen by GC/MS in postmortem case involving medical resuscitation).

1.4.3.6. When mass spectrometry is used in SIM for the simultaneous quantitation and identification of an analyte, retention time match is required and the use of at least two analyte qualifying ions and one internal standard qualifying ion is preferred. Acceptance criterion for ion ratios is 20% or 2 SD relative to that of averaged calibrators or controls. However, it is recognized that some ion ratios are concentration dependent and that comparison to a calibrator or control of similar concentration may be necessary.

1.4.3.6.1. With some MS assays, ion ratios may be more dependent on concentration; therefore ion ratio acceptance ranges may not be static across the calibration curve and will be determined by method validation and documented in the SOP.

1.4.3.7. Following GC/MS and GC/NPD screening analysis (of the same aliquot), it is common practice for this laboratory to report non-toxicologically relevant drugs (e.g. low levels of caffeine, nicotine, and lidocaine) as “Present”. If the reviewing toxicologist determines that the concentration of one or more of these drugs is approaching toxicologically relevant concentrations, further testing will be performed.

1.4.3.8. The laboratory does not report any controlled substance without a second aliquot analysis/confirmation with the following exceptions:

1.4.3.8.1. The controlled substance is a metabolite of a parent drug that was detected in the screen.

1.4.3.8.2. The controlled substance is a chemical precursor or byproduct in the synthesis of an illicit substance (e.g., 4-ANPP) and the final synthesis product (e.g., fentanyl) is present.

1.4.3.8.3. In the rare occurrence of insufficient specimen for further analysis.

1.5. Guidelines for Batch Analysis

1.5.1. In order to maximize efficiency within the toxicology laboratory, it is common to group specimens into batches. The following are general guidelines for batch analysis, unless otherwise specified in a method SOP.

1.5.2. The transfer, handling and aliquoting of specimens are documented with electronic chain-of custody (COC) as well as an aliquot COC worksheet. Each load worksheet also contains the unique OCME Toxicology load number which is filed by assay type, where the hardcopy of the original raw data (including chromatograms of calibrators/controls, calibration curves, instrument sequence lists, etc.) shall be stored (TOX-P17).

1.5.3. Each load must contain a sufficient number of controls to monitor the performance of the assay however the total number of controls will depend on the size of the load and the nature of the tests. A minimum of one positive and one negative control is required.

1.5.4. For quantitative analysis (See 2.6), each batch of specimens should include a calibration curve (minimum of 3 points for volatiles and 4 or more points for all others) and, at minimum, one matrix specific positive (See 2.9) and one matrix specific negative control (See 2.10 if matrix-matched QC specimens are not available).

1.5.4.1. Case samples should be bracketed by acceptable calibrators and controls.

1.5.4.2. The identity and position of each vial in the autosampler must be verified by an independent analyst with the autosampler sequence list prior to beginning the

sequence. This vial verification is documented with initials and date on the sequence list.

1.5.4.3. If a batch analysis fails to meet the QA/QC acceptance criteria, a copy of each sample report with a written explanation of why the data is unacceptable and/or a NTF shall be included in each case file.

1.5.4.3.1. At minimum, a copy of the Chain of Custody and the reason for the batch failure (NTF) shall be included in each case file.

1.5.4.4. For quality assurance purposes, each batch analysis must be reviewed by an independent analyst and this review is documented with a signature and date on the load worksheet and initials and date on each sample report. The batch analysis review process includes a review of the following items:

1.5.4.4.1. Assignment sheet
1.5.4.4.2. Comments or note to file (if applicable)
1.5.4.4.3. Load summary
1.5.4.4.4. Specimen worklist
1.5.4.4.5. Chain of custody (Specimen)
1.5.4.4.6. Aliquot chain of custody
1.5.4.4.7. Standard and control worksheet
1.5.4.4.8. Verified sequence list (if applicable)
1.5.4.4.9. Sequence summaries/calibration reports
1.5.4.4.10. Calibration data
1.5.4.4.11. Blank matrix data
1.5.4.4.12. Control data
1.5.4.4.13. Specimen data

1.5.4.5. Once the load summary review process is complete, the standard and control worksheet and aliquot COC (if applicable) is scanned (pdf format) by the documentation specialist (or proxy) and saved in the load folder containing the raw data.

1.5.4.5.1. A copy of each sample report will then be filed in the appropriate case folder.

1.6. Guidelines for Mass Spectrometer Peak Identification

1.6.1. Experience in interpretation of mass spectra and retention time data is key to making correct peak identifications. Less experienced analysts are encouraged to seek help from senior chemists and/or toxicologists if they have doubts about the identity of a chromatographic peak.

1.6.2. The analyst/toxicologist should assess the spectral match for a peak to be identified for the following mass spectral characteristics using the following questions:

1.6.2.1. Does the mass spectral quality match exceed 70%? (Note: This is only a starting point as drugs may have similar mass spectra with relatively little to distinguish it from another (lack of spectral character).)

1.6.2.2. Does the mass spectral fragmentation pattern have appropriate relative abundance of each ion?

1.6.2.3. Is the base ion present?

1.6.2.4. Is there a molecular ion?

1.6.2.5. Are there characteristic and/or unique ions present?

1.6.3. In addition, retention time may be used to differentiate drugs with similar mass spectra (e.g. amitriptyline, cyclobenzaprine and doxepin). In order for a compound that is present in the standards to be identified in a case, it needs to have a matching retention time with the analyte in the standard that is run within the same batch. For less frequently detected analytes and analytes not present in the standard drug mixes, use of the TC4 RRT/RI (S:\toxicology\analyst\access\toxdata) database can help determine whether or not the peak to be identified is eluting in the correct elution order relative to peaks of known retention time (e.g. peaks present in the standards).

1.6.4. Caveats: Although the computer-based match quality value for most drugs and drug metabolites is usually greater than 75-80%, the match quality value of some may be less. The lower percentages may be explained by answering the following questions:

1.6.4.1. Does the mass spectrum have a low molecular weight base peak (e.g. 44 or 58) and lacking characteristic and/or unique ions?

1.6.4.2. Is the concentration of analyte in the specimen extract very high or very low?

1.6.4.3. Is there a co-eluting analyte?

1.6.4.4. Is there noise or GC column bleed contributing ions to the mass spectrum?

1.6.5. In the case of a low quality match but reasonable retention time and one or more explainable factors from the above questions, the analyst/reviewer should assess the spectrum in the following way:

1.6.5.1. The library match for the unknown must contain at least 5 or more diagnostic ions when the match quality is less than 50%. If fewer than 5 diagnostic ion fragments are present, match criteria will be at the discretion of the reviewing toxicologist in light of all data derived from the case. Analysts should consider these identifications as tentative and label them accordingly on the chromatogram (Consistent with (c/w)).

1.6.6. Final Assignment of Analytes

1.6.6.1. The final assignment of analytes requires appropriate assessment of: the mass spectrum, retention time and information from the case (e.g. history, prescription product, presence of unique metabolites) and is the purview of the reviewing toxicologist. In addition, the presence of unique metabolites and the reported case history, which may include a tabulation of suspected drugs, may assist in the assignment of analytes.

1.6.7. Organic Bases and A/N processing (SOP 054):

1.6.7.1. To complete the task of peak assignment, label all known peaks on the NPD/FID and MS chromatogram. This should include:

1.6.7.2. For NPD/FID chromatograms, peak(s) of known origin and present in the standards are labeled by the software as part of quantifying the case. Because peaks may have the same RT or elute within the same RT window, the analyst is responsible for “q-deleting” the misidentified peaks flagged by the software on the NPD chromatograms. For FID chromatograms the analyst will annotate the misidentified peaks as none detected “ND” in the margin. Otherwise hand-label the peaks.

1.6.7.3. For MS chromatograms, peak(s) of known origin should be annotated (hand-labeled) with the name of analyte.

1.6.7.4. For both NPD and MS chromatograms:

1.6.7.4.1. peak(s) of unknown identity and/or origin – annotate with “NI”

1.6.7.4.2. peak(s) attributed to extraction artifacts- annotate with “Ø”

1.6.7.4.3. peak(s) attributed to decomposition products- annotate with “c/w decomp”

1.6.7.4.4. peak(s) with a tentative identification- annotate with the “c/w” and analyte name. Alternately, for peaks that appear to be related to a known analyte the peak should be annotated with c/w “analyte name” related. This indicates that the identification is tentative and the analyte will not appear on the final report.

1.6.7.5. Transfer the results of the drug screen into Toxlog according to reporting criteria as outlined in SOP 102 and 103.

1.7. **Proficiency Testing (PT) Guidelines**

1.7.1. The laboratory participates in the following PT programs:

1.7.1.1. CAP AL1 (volatiles and ethylene glycol only)
1.7.1.2. CAP FTC
1.7.1.3. CAP T
1.7.1.4. CAP VH
1.7.1.5. PA Dept of Health (volatiles only)
1.7.1.6. CAT
1.7.1.7. Inter-Lab Tissue
1.7.1.8. NHTSA (volatiles only)

1.7.2. Accessioning and test assignment

1.7.2.1. The QA/QC chemist (or proxy) will fill out a Toxicology Request Form for each PT specimen as applicable.

1.7.2.2. The following drug screens will be requested (when applicable):

1.7.2.2.1. Volatiles
1.7.2.2.2. Ethylene Glycol
1.7.2.2.3. Organic Base Screen
1.7.2.2.4. LC Screen
1.7.2.2.5. Acid/Neutral Screen
1.7.2.2.6. Salicylates

1.7.2.3. As appropriate, PT specimens in non-conforming containers shall be transferred to 20mL scintillation vials to better appear as ME specimens.

1.7.2.4. The PT specimens, along with accompanying TRF are passed to the evidence technician for accessioning.

1.7.2.5. In order for the Medical Examiner Information System (MEIS) to differentiate between PT specimens and actual case specimens, the following criteria are entered:

1.7.2.5.1. Date of death prior to 1971.
1.7.2.5.2. County of death = Hyde County.

1.7.2.6. Once entered into MEIS, PT testing is performed by toxicology personnel along with routine case work.

1.7.3. Results

1.7.3.1. OCME reporting criteria (e.g. significant figures, units) will be superseded by the PT provider reporting instructions. As such, results on the OCME toxicology report may differ from the results reported to the provider.

1.7.3.2. Only results that have been completed and accepted by a toxicologist as meeting reporting criteria by the due date will be submitted to the PT provider.

1.7.3.3. In the rare instance when quantitative testing is not complete by the PT due date, then results may be reported qualitatively provided they meet acceptable laboratory guidelines for qualitative reporting. Once complete, the quantitative results will be compared to the group statistics when the PT score sheet is received by the laboratory. A NTF explaining the circumstances surrounding the delayed results and final evaluation of the results will be completed and stored with the PT score sheet.

1.7.3.4. PT results are reviewed and a "Review of Proficiency Testing" form (Policy Tox-P9) is completed for each set of survey results. The form is signed by the QAQC Chemist and the Chief Toxicologist.

1.7.3.5. The laboratory results are assessed in the following manner:

1.7.3.5.1. Quantitative results acceptable or unacceptable as deemed by the PT provider (usually ± 2SD of group mean although other criteria may be used by specific PT providers).

1.7.3.5.2. Qualitative results acceptable or unacceptable as deemed by the PT provider (e.g. absence of "false" positives and "false" negatives).

1.7.3.5.3. Quantitative results acceptable or unacceptable as deemed by OCME.

1.7.3.5.3.1. Volatiles for concentrations ≤ 100 mg/dL OCME result is required to be with 10 mg/dL of the group mean, for concentrations > 100 mg/dL OCME result is required to be ± 7.5% of the group mean.

1.7.3.5.3.2. Drugs: OCME result will not exceed ± 20% of the group mean.

1.7.3.5.4. Qualitative results acceptable or unacceptable as deemed by OCME: All analytes for which the laboratory has screening and confirmation methods will be detected and reported. Analytes that the laboratory does not have screening and confirmation methods or does not test for or report by policy (e.g. THC and THC-COOH) but are deemed an intended response by the PT provider will receive acknowledgement as such on the "Review of Proficiency Testing" form but do not require further investigation.

1.7.3.6. All results deemed "unacceptable" by the PT provider or by internal lab criteria will be investigated, as appropriate. Investigation documentation along with a corrective action report (TOX-P11) will be filed along with the PT score sheet.

1.7.3.7. PT results will not be discussed with the staff from other laboratories prior to final grading by the PT provider.

1.8. Non-conforming work – Deviation from SOP

1.8.1. Minor deviations (change in sample size, addition of a low or high calibrator to extend the reporting limit) from the SOP are permitted in rare circumstances and as long as they are performed and evaluated in a scientifically sound manner. All deviations must be approved prior to the analysis and must include a plan that will evaluate the results.

1.8.2. The change to the assay will be detailed in a "Special Analysis Note-to-File" that will be completed, approved, and signed by a Toxicologist or the QA/QC Chemist and placed in the folder prior to analysis. The note to file will detail the planned changes to the assay and the criteria for acceptance of the assay. Some examples are listed below:

1.8.3. Extending the reporting range of the assay:

1.8.3.1. The reporting range of an assay may need to be extended in rare circumstances mostly due to low sample volume. New calibrators at ½ of the low standard or 2x the high standard may be used. The batch will include a QC that tests the validity of the new standard. At the low end of the curve the QC will be 20% higher than the new LOQ. At the high end of the curve the QC will be 5% lower than the new ULOL.

1.8.4. Review includes verification of the acceptability of the new qc's and formal documentation on the "Special Analysis Note-to-File"

2. Quality Control

2.1. Definitions

2.1.1. Calibrator: An analytical standard of known concentration used to establish the relationship between concentration of analyte and the corresponding instrument response.

2.1.2. Continuing Calibration Check (CCAL): A reference specimen (often a mid-level calibrator) that is analyzed periodically to establish that the instrument calibration is being maintained.

2.1.3. Control: An analytical standard of known concentration used to test the accuracy of the calibrator (typically prepared from a different lot of source material).

2.1.4. Linear range: For most chromatographic assays, the Limit of Quantitation (LOQ) and Upper Limit of Linearity (ULOL) are administratively defined in terms of the

concentration of the lowest and highest calibrators used in the calibration response curve, respectively.

2.1.5. Limit of detection (LOD): The lowest concentration at which an analyte is determined to be present in a sample. Can be administratively defined (e.g. ½ the LOQ), calculated using the slopes and y-intercepts of multiple calibration curves (8.4.6), or determined using serial dilutions to ascertain the lowest concentration the analyte exhibits all chromatographic acceptance criteria (minimum of 5 duplicate analysis).

2.1.6. Decision Point: An administratively defined qualitative lower reporting limit (similar to a screening cutoff). The purpose of the Decision Point is to determine whether the compound needs to be confirmed or quantified. Compound concentrations equal to or greater than the Decision Point shall be confirmed and/or quantified. A compound may be confirmed or quantified below the Decision Point at the discretion of the reviewer upon considering all available information in the case. As with all analyses, the reviewer must ensure that all qualitative acceptance criteria have been met when working under the Decision Point.

2.1.7. Limit of Quantitation (LOQ): The LOQ is typically the lowest calibrator concentration included in the calibration response curve. To include the LOQ in calibration response curve, the LOQ, must have acceptable ion ratios, if applicable, and must calculate within 25% of target concentration. If the LOQ does not meet these criteria, then the new LOQ is the lowest calibrator that satisfies these criteria.

2.1.8. Upper Limit of Linearity (ULOL): The ULOL equals the highest calibrator concentration included in the calibration response curve with acceptable ion ratios, if applicable, and whose calculated concentration falls within 10% of target concentration. If the ULOL does not meet these criteria, then the new ULOL is the next highest calibrator that satisfies these criteria.

2.2. **Calibrators and Controls (See SOP-010 for preparation details)**

2.2.1. Whenever possible, calibrators and controls are to be prepared from different lots of stock material.

2.2.2. Typically standards are given an arbitrary expiration date of 1year (shorter expiration dates may be assigned per analytes COA or due to previously observed performance).

2.2.2.1. A standard may remain in use beyond its expiration date if, when analyzed against a newly prepared standard (or other published criteria (A1%)), the measured concentration falls within ±15% of target.

2.2.2.2. An analytical batch must have, at least, a calibrator or control that is within its expiration window.

2.2.3. Preparation of calibration and/or positive control standards used in quantitative assays are to be prepared by a Toxicologist, QA/QC chemist, laboratory supervisor, or an appointee who has been trained and approved by the above listed personnel.

2.2.3.1. All calibration and/or positive control standards used in quantitative assays must be approved by the QA/QC chemist prior to use (see section 7.5)

2.2.4. Preparation of internal standards and calibration and/or positive control standards used in qualitative assays may be prepared by any trained technician or chemist.

2.3. Qualitative Colorimetric Tests (Color Tests)

2.3.1. A minimum of one positive control and one negative control is required for all qualitative colorimetric tests.

2.3.2. For a color test to be considered successful, the following conditions must be met:

2.3.2.1. A positive result (color change) must be observed in the positive control(s).

2.3.2.2. A negative result (no color change) must be observed in the negative control(s).

2.3.3. If the matrix of a case specimen differs from the control matrix, an additional aliquot of the case specimen will be taken and spiked with a positive control.

2.3.3.1. The spiked aliquot must demonstrate a positive result (color change) for the specimen to be considered suitable and results reported.

2.4. Other Non-Chromatographic Assays

2.4.1. Assays implementing instrumentation specific to analyte(s) of interest (Carbon Monoxide – UV-VIS, Glucose/Electrolytes – pHOx Ultra analyzer) have unique QC acceptance criteria that is outlined in detail in the assay SOPs.

2.5. Qualitative Chromatographic Assays

2.5.1. Analyze a minimum of one negative and at least one positive control/reference standard along with unknowns in each chromatographic assay. If only one positive control is included in the batch, the concentration must be at the decision point for the assay.

2.6. Validated Quantitative Assays

2.6.1. Prepare a minimum of three different calibrators (Volatiles) or a minimum of four different calibrators in each quantitative assay or as specified in the assay specific

SOP. The concentration of the calibrators should be such that they bracket the anticipated concentration of the unknown specimen(s).

2.6.2. Prepare a response curve of area of analyte to area of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot. It is acknowledged that some assays are inherently non-linear (e.g. LCMS) and the use of weighted or quadratic models may be necessary and appropriate, and should be verified using low and high controls. The response curve and determined unknown specimen concentration(s) are generated using data processing software.

2.6.3. The calculated coefficient of determination (r^2) for the curve. For most applications, an r^2 of greater than or equal to 0.992 is acceptable (unless otherwise indicated in a specific assay SOP).

2.6.4. Evaluate the curve by calculating calibrator concentrations against the curve. Values within ± 20% (25% for the LLOL) from the target calibrator concentration are acceptable.

2.6.5. For loads with greater than 20 case specimen injections, a mid-level calibrator will be re-injected mid-way through the analytical run and must pass within 20% of target value.

2.6.5.1. If the mid-run calibration check falls outside of acceptance limits, no results shall be reported from that run (for that analyte) and all cases will be repeated.

2.6.6. Assays for which validation is pending: Specimens may be analyzed using Non-matched Matrix QC Protocol (2.10).

2.7. **Calibration curve data point inclusion guidelines (quantitative assays):**

2.7.1. At least 3 calibrators must be included in the calibration curve.

2.7.2. No more than 1 calibrator may be removed from the calibration curve.

2.8. **Calibration curve point exclusion guidelines:**

2.8.1. In order to manually exclude a point from a calibration curve, at least one of the following criteria must be met:

2.8.2. The calculated concentration of a calibration level exceeds 20% of the target concentration unless otherwise stated in SOP.

2.8.3. The exclusion of a calibration level improves the R^2 by 0.005 (e.g. 0.993 to 0.998) or from failing (<0.992) to passing (≥0.992).

2.8.4. The chromatographic peak of a calibration level does not meet acceptance criteria (see 2.12)

2.8.5. Internal standards: Internal standards are required for chromatographic quantitative assays.

2.8.5.1. Use an internal standard with similar extraction, derivatization, and chromatographic properties to the analyte(s) of interest.

2.8.5.2. The use of stable isotope internal standards for GCMS and LCMS is encouraged but not required since well-chosen non-deuterated internal standards may give similar performance.

2.8.5.3. The internal standard recovery as measured by peak area or ion abundance should be monitored for calibrators, controls and case specimens.

2.8.5.4. To ensure quantitative and qualitative results, the internal standard (IS) recovery of the samples should be similar to that of the calibrators and controls. During data review, the examiner and reviewer should be mindful of these recoveries and remediate accordingly if it appears that the IS recovery of the sample falls below half or greater than twice (50%-200%) that of the average IS recovery of the calibrators and controls. Remediation actions include the following:

2.8.5.4.1. For analysis run in duplicate, if only one of the sample pair exhibits IS recovery outside acceptance limits and the target analyte(s) calculations are comparable (± 25%), the data may be accepted.

2.8.5.4.2. Reinject the affected case sample followed by the appropriate QC specimen to determine whether or not the low or high recovery was due to a poor injection (see section 2.14.1).

2.8.5.4.3. Reanalyze the case specimen.

2.8.5.4.3.1. Upon reanalysis, if the internal standard recovery still falls outside approximately 50 - 200% of the average internal standard recovery:

2.8.5.4.3.2. The case specimen may be reanalyzed using the Non- Matched Matrix Protocol (2.10).

2.8.5.4.3.3. The case specimen may be reanalyzed using a different internal standard. This major deviation and exception shall be authorized by the Chief/Deputy Chief Toxicologist and documented in the case file with a NTF.

2.8.5.4.3.4. The drug may be reported as “present” if its identity has been confirmed. This deviation and exception shall be authorized by the Toxicology Supervisor or Toxicologist. The reason for reporting “present” shall be documented in the case file.

2.8.5.4.3.5. The case specimen may be reported as “specimen unsuitable” for the particular assay. This deviation and exception shall be

authorized by a reviewing chemist, documented on the data file and a result comment added in the toxicology database to appear on the final report.

2.9. **Controls:**

2.9.1. Controls in quantitative assays may be purchased or prepared in-house. In-house prepared controls should be prepared from a different manufacturer and/or different lot of standard material than used in calibrators. If this is not possible, controls should be prepared from a different weighing or dilution from the purchased stock calibrators, preferably by another analyst. Results from quantitative quality control materials are recorded in control charts (Levey Jennings) to readily detect trends such as deterioration of reagents, calibrators and controls.

2.9.2. For assays that are run ≥ 10 times per month, the control data will be charted to include the mean, 1, 2, and 3 standard deviations.

2.9.3. ≥ 2 QC failures in a row (for a given analyte) warrant an investigation

2.9.4. Control data will be reviewed monthly by the QA/QC Chemist or other senior chemist (Chemist II or higher). The review and any investigations and findings will be documented via note to the electronic control chart.

2.9.5. Analyze a matrix specific negative control (blank) and at least one matrix specific positive control (QC) with each quantitative procedure.

2.9.5.1. If a matrix specific blank matrix is not available for a case specimen, follow the laboratory's Non-matched Matrix Protocol (2.10).

2.9.6. The target analyte concentrations of positive controls must fall within the linear range of the assay

2.9.7. For assays in which the concentration range of the calibration is ≤ 1 order of magnitude, one Low-Mid-level positive control will be used.

2.9.8. For assays in which the concentration range of the calibration is ≥ 1 order of magnitude, two positive controls will be used (low and high).

2.9.9. The laboratory will maintain control charts (Levey Jennings) of positive controls on the shared drive.

2.9.10. For assays run < 10 times a month but > 5 times per year

2.9.10.1. Positive control range is ± 20% from the target concentration unless otherwise noted in the method SOP.

2.9.10.2. If the concentration of a positive control (for a given analyte) should fall outside the acceptance limits stated above, the analysis for all cases in the batch, containing that analyte, will be repeated.

2.9.10.2.1. The analyte may be reported qualitatively with approval of a reviewing chemist.

2.9.11. For assays run <5 per year

2.9.11.1. Minimum of 6 point curve will be utilized.

2.9.11.2. A minimum of two QC levels will be tested, one at both the low and high end of the calibration curve will be evaluated using the criteria outlined in 2.9.10.1

2.9.12. The negative control must indicate that the analyte of interest is not detected.

2.9.12.1. If a signal is present, the detected area ratios of the quantifying ion must be below 10% that of the lowest calibrator for the same analyte.

2.9.12.2. If a signal is present with an area ratio above 10% that of the lowest calibrator, the Toxicology Supervisor should be notified.

2.9.12.3. The negative control may be re-injected at the end of the run following a solvent blank

2.9.12.3.1. If no analyte is detected in the re-injected negative control, this indicates carryover as the cause of the anomalous result.

2.9.12.3.2. Any unknown specimens positive for the analyte in question that were analyzed immediately after a specimen that was negative for the analyte may be reported as no carryover is possible following a negative sample.

2.9.12.3.3. If the target analyte is detected in the re-injected blank, a conclusion can be made that the blank matrix is contaminated. All case specimens containing that analyte must be repeated. The specific lot of blank matrix used will subsequently be quarantined and re-screened for target analytes.

2.10. **Non-Matched Matrix QC Protocol**

2.10.1. Serum, plasma, vitreous humor, and “bloody fluid” are considered equivalent to blood for the purposes of this procedure – no matrix spike required for these specimens. **Note – Fluid (not otherwise specified) <u>does</u> require a matrix spike.**

2.10.2. Analyze a matrix spike in addition to the specimen:

2.10.2.1. Aliquot the specimen in duplicate (as stated in the assay specific SOP).

2.10.2.2. Take a third aliquot of the specimen (1:1) and spike it with the QC standard at a similar concentration as the specimen being analyzed (if known).

2.10.2.3. If the specimen concentration cannot be estimated from historical case data spike with QC at the concentration described below:

2.10.2.3.1. Brain, muscle, misc. fluids – spike with low QC level.
2.10.2.3.2. Bile and gastric – spike with high QC level.

2.10.2.4. The calculated concentration of the matrix spiked specimen, minus the added QC amount, is compared to the average of the analyzed specimens.

2.10.2.4.1. If the calculated concentration of the matrix spiked specimen (minus the QC) falls within 25% of the calculated average of the duplicate specimens, no significant matrix effect is demonstrated and the result may be reported using the assay's reporting guidelines (as outlined in assay specific SOP).

2.10.2.4.2. If the calculated concentration of the matrix spiked specimen falls outside of the QC acceptance limits, a matrix effect maybe present and the result can be reported qualitatively or reanalyzed using the Standard Addition Protocol (2.11).

2.10.2.5. It is important that the standard addition in the above procedure be large enough to provide an obvious additive affect, but not so large as to make the resident analyte's presence in the spiked specimen insignificant. If the spiked concentration is not between 50% and 250% of the targeted analyte's measured concentration in the unknown specimen, the procedure shall be repeated with an appropriate spike concentration.

2.11. **Standard Addition Protocol**

2.11.1. This procedure is limited by available sample volume and must be approved by a Toxicologist.

2.11.2. Following the assay specific SOP, prepare a calibration curve, blank, and QC using the specimen to be analyzed as the matrix.

2.11.3. Extract and analyze the specimens following the assay specific SOP.

2.11.4. After analysis, construct a calibration curve.

2.11.4.1. Evaluate the blank and QC against the calibration curve.

2.11.4.2. If the calculated QC amount falls within acceptance limits for the assay, proceed with next step, if not report the result qualitatively.

2.11.5. Calculate the concentration of target analyte in the specimen:

2.11.5.1. Using the calibration curve equation, enter 0 for X and solve for Y.

2.11.5.2. The concentration of the target analyte = the y-intercept multiplied by -1.

2.12. Chromatographic and Mass Spectral Quality Control

2.12.1. Chromatographic quality control: Some toxicology casework may contain multiple drugs or co-eluting decomposition products that may prohibit adherence to some of the following chromatography guidelines. Exceptions shall be documented in NTFs or on chromatograms.

2.12.1.1. Retention Time: Retention times for both analyte and internal standard must be within ± 2.0% (GC Methods) and ±3.0% with a 7.2 second window minimum (LC methods) of the retention time obtained from the average of the analytical run

2.12.1.2. Peak Resolution: To the greatest extent possible, chromatographic peaks used for quantitative analyses should be resolved from interfering peaks such that the valley between adjacent peaks are no greater than approximately 10% (by visual inspection) of the peak height of interest.

2.12.2. When poor instrument performance results in unacceptable chromatography, low or inconsistent recovery, shift in retention time(s), or other problem resulting in unreliable data, the Instrument Chemist should be notified immediately.

2.12.2.1. It may be necessary to re-inject the specimens after instrument maintenance is performed or on a different instrument as appropriate.

2.12.2.2. Documentation in the form of a NTF is required to explain any issues with instrumentation and steps taken to remediate the problem.

2.13. Selected ion monitoring (SIM) identification:

2.13.1. When SIM is used for identification of an analyte, whether as part of a quantitative procedure or not, retention time match is required and the use of at least two analyte qualifying ions (one internal standard qualifying ion) is required.

2.13.2. For analytes with limited qualifying ions, it is minimally acceptable to use one qualifying ion in addition to the target ion.

2.13.3. If selected ion monitoring is being carried out for quantitation only, and the analyte has already been identified separately, the above criteria may be considered desirable rather than mandatory.

2.13.4. Acceptance criteria for ion ratios are ± 20% (or as specified in the SOP) the average ratio from a mid-level calibrator, (Cal4 in a 6–point curve, Cal3 in a 5-point curve).

2.13.4.1. It is recognized that some ion ratios are concentration dependent and that an average ratio for the calibration curve or comparison to a calibrator of similar concentration may be necessary rather than the. This exception shall be documented in the associated SOP.

2.14. LC/MS/MS Identification

2.14.1. LC/MS – Ion Trap

2.14.1.1. Chromatogram

2.14.1.1.1. Peaks must be symmetrical (Gaussian shaped).

2.14.1.1.2. Retention time must not deviate outside ± 0.10 minutes of target, based upon the retention time of the calibrators and controls.

2.14.1.2. Mass spectroscopy

2.14.1.2.1. The mass spectrum for each detected analyte in an unknown specimen shall be compared to the mass spectrum of that same analyte in the matrix matched quality control (QC High) sample from the same load. All major ions shall be present and ion ratios consistent with that of the QC sample via visual inspection.

2.14.1.2.2. The mass spectra of each analyte shall be consistent from load to load. Below are reference spectra to be used to verify load acceptance. Each analyte must pass the above criteria for the appropriate matrix type for an analyte to be considered “present”.

2.14.2. LC/MS/MS (Triple-Quad) is not used for analyte identification at this time.

2.15. Reinjection criteria and documentation

2.15.1. Occasionally, there may be situations in which calibrators, controls and case samples need to be reinjected on an instrument. Common reasons for reinjection include the following:

2.15.1.1. Misinjection by the autosampler

2.15.1.2. Inconsistent ion ratio and/or retention time

2.15.1.3. Poor chromatography or interference: The sample is reinjected to determine if the cause is due to the analytical instrument or sample/sample preparation.

2.15.1.4. Poor analyte or internal standard recovery: The vial is reinjected to determine whether poor recovery is due to the injection or sample preparation.

2.15.1.5. Sample overload: In a confirmation assay this would most often be above the limit of quantitation and necessitate repeating the extraction at dilution. In assays where the specimen is simply diluted, such as an ID of physical evidence, the sample may be diluted and reinjected.

2.15.1.6. Carryover: Carryover may occur due to the use of automated GC and LC/MS injection systems or due to the extreme range of drug concentrations detected in toxicological specimens. Often, in GC and LC analysis, an unknown specimen may contain a very high concentration of a target analyte (relative to the linear range of the assay). Due to the extreme concentration, the analyte may not be fully cleared from the injector, inlet, tubing, or column prior to the injection of the next specimen. As a result, that same analyte may appear in the subsequent chromatogram(s) producing a false positive. An analyst should be suspicious of carryover in specimens following a case with a large peak and/or when same analyte is detected in multiple chromatograms in a row, especially if the peak response decreases over time. Carryover can also occur during the extraction process, when a pipette used to aspirate or transfer extracts becomes contaminated, transferring a small concentration of target analyte to subsequent test tubes or vials. Regardless of cause, extreme caution is warranted when carryover is suspected and requires senior chemist notification, guidance and review of the analytical results. Appropriate actions may include reinjection with solvent blanks between specimens or reanalysis of some or all of the specimens. The senior chemist shall document their review of the carryover root cause analysis and the appropriate response on a NTF to be included with each affected sample report and affected individual case files. The following procedures are used to help detect/prevent carryover and mitigate the consequences.

2.15.1.6.1. Method Validation (see section 8.4.9)

2.15.1.6.1.1. Each assay utilized in routine casework is evaluated for the carryover threshold of each target analyte. If any analyte is present at or above this threshold, the following specimen will be scrutinized for the possibility of carryover.

2.15.1.6.2. Analyte Confirmatory Procedures (see section 1.4)

2.15.1.6.2.1. Nearly all of the analytes detected within the laboratory's various screening assays are then reanalyzed using a different technique to verify their presence and concentration. This procedure helps to mitigate the impact of carryover occurring in a screening procedure that could otherwise result in the reporting of a false positive result.

2.15.1.6.3. Duplicate analysis during confirmation ensures that carryover can be further evaluated/detected as the results will not pass reporting criteria because the calculated values will not match.

2.15.1.6.4. Negative controls (see section 2.9.12)

2.15.1.6.4.1. Negative controls (method blanks) are typically analyzed following a calibrator representing the ULOQ of the assay. A method blank failing acceptance criteria (often due to carryover) may indicate a

problem with the instrument which must be remedied prior reinjection or injection of another batch.

2.15.1.6.5. Instrument Maintenance (SOP 061 & SOP 071)

2.15.1.6.5.1. Routine instrument maintenance is critical to assist in the prevention of carryover. Timely replacement of syringes, septa, inlet liners, tubing, analytical columns, and other items often can remove the root cause.

2.15.1.6.6. Multiple Stages of Review (sections 2.18 & 2.19)

2.15.1.6.6.1. All analytical data undergoes multiple stages of review. This allows for multiple individuals to scrutinize the data to find analytical anomalies (such as carryover) before any results are reported.

2.15.1.6.6.2. Reviewers have the responsibility to assess all the information from the case history, analytical results, and/or confirmations prior to certifying a result for reporting. Options available to the reviewer include analysis of a second specimen, reanalysis of the original specimen, or in the case of trace amounts of analyte, designating the result as consistent with carryover and suppressing the reporting of the analyte in question.

2.15.1.7. Reinjected sample(s) (unless they are analyzed immediately following the completion of the load), must be followed by reinjection of either the duplicate case sample or matrix-matched calibrator or control. All samples must meet the assay's QA/QC criteria as outlined in the SOP.

2.15.1.8. It may also be necessary to reinject the entire load. The load workbook shall be re-saved with an "r" at the end of the file name and all data files saved into the new workbook.

2.16. **Documentation of Reinjection.**

2.16.1.1. Document on the original chromatogram that the original injection was unacceptable using language such as "Not used due to (reason for reinjection)" or "Misinjection".

2.16.1.2. Document on the reinjected chromatogram any action taken prior to reinjection, e.g. "reinjection – dilution of sample extract."

2.16.1.3. The data file name of the reinjected vial is the same as the unacceptable data file name except that it is appended with "r" for "reinjection".

2.16.1.4. If the entire load was reinjected, a NTF shall be included with the load paperwork explaining why the initial injection was unsuccessful and where the original data can be found.

2.17. **Criteria for Reporting Toxicology Case Results**

2.17.1. Drug reporting guidelines: Report drug concentrations in mg/L or mg/kg on the final report unless otherwise stated in the assay specific SOP.

2.17.2. All results are truncated (not rounded). For samples analyzed in duplicate, duplicate results must agree within 25% ((maximum result divided by minimum result), minus 1, multiplied by 100) and the average of the two results shall be reported (except ethanol – SOP-101).

2.17.2.1. A specimen will be repeated up to three times per analytical method if the 25% max/min criterion is not met. If after the third analysis comparable results have not been achieved, the average of all the results will be taken, truncated, and reported with the prefix "(approximately)". Documentation of the averaging of multiple analytical runs will be made on the final data file and the following result comment added in the toxicology database to appear on the final report: "Specimen matrix effects prevent exact measurement of "drug of interest". Amount reported is the average of "number of results averaged" measurements".

2.17.3. If multiple dilutions are analyzed, report the least dilute sample that falls within the linear range of the assay. If all dilutions fall within the linear range of the assay, the truncated average will be reported.

2.17.4. If biological fluids or tissues are diluted prior to the analysis, multiply the analytical results truncated to the same number of significant figures as the calibrators by the dilution factor (e.g. methadone 0.54 mg/L run with 100 fold dilution should be reported as 54 mg/L).

2.17.5. Report blood or biological matrix drug concentrations greater than or equal to 0.10 mg/L with two significant digits (e.g. 0.12 or 1.2 mg/L (mg/kg for weighed aliquots)).

2.17.6. Report toxicologically significant blood or biological fluid drug concentrations less than 0.10 mg/L with two significant figures (e.g. 0.015 mg/L) and a maximum of three decimal places (e.g. 0.006 mg/L).

2.17.7. Concentrations of drugs found in urine specimens may be reported qualitatively or quantitatively (if the matrix has been previously validated), however, it should be noted that the concentration provides little interpretive value.

2.17.8. A drug may be reported as "Present" for a number of reasons including specific reporting guidelines in a method SOP or quantitative procedures were not performed or available or a quantitative procedure was performed but acceptance criteria were not satisfied and reanalysis is not possible or practical.

2.17.9. To report a drug as "Present," its identity must be confirmed by secondary analytical technique, except in those conditions already discussed previously in (1.4.3.7).

2.17.10. "Present" may be used to report the presence of drugs in different tissues or fluids for consistency and support of analytical findings (e.g. report a case with

postmortem methadone findings of blood 1.3 mg/L, liver 20 mg/kg and urine "present").

2.17.11. Report drug as "Less than __"

2.17.11.1. The confirmed analyte has had a quantitative procedure performed and the drug concentration was at or above the LOD but less than the LOQ.

2.17.11.2. Report as "Less than (LOQ concentration)."

2.17.11.3. If a dilution factor was used in the analysis multiply the dilution factor by LOQ to use for the reported LOQ.

2.17.12. Report drug as "Greater than __"

2.17.12.1. The confirmed analyte has had a quantitative procedure performed but the drug concentration was greater than the ULOL of the assay and reanalysis is not possible, practical, or not needed for interpretation.

2.17.12.2. Report as "Greater Than (ULOL concentration)."

2.17.12.3. If a dilution factor was used in the analysis, multiply the dilution factor by ULOL to use for the reported ULOL.

2.17.13. Report as "None Detected."

2.17.13.1. Absence of target analyte.

2.17.13.2. Analyte not confirmed.

2.17.13.3. A quantitative analysis was attempted and the drug did not satisfy confirmation criteria or was below the LOQ or LOD.

2.18. Batch Review

2.18.1. Batch acceptance criteria are outlined within the SOP for each assay.

2.18.1.1. The first stage of review is a self-review by the laboratory Chemist or Chemistry Technician who performed the batch analysis.

2.18.1.2. The second stage of review is performed by a qualified Chemist or Toxicologist who has been trained on the review process for the particular assay.

2.18.1.3. The first and second stage of review involves initialing the load checklist, thus certifying the results from that batch.

2.19. Procedure for Case Review and Toxicology Report Certification

2.19.1. Introduction: The reporting of accurate and forensically defensible toxicology results is paramount to the mission of the NC-OCME toxicology laboratory. This protocol is a part of the laboratory Quality Assurance program focused on the review and evaluation of the provisional toxicology report and toxicology folder data prior to report certification.

2.19.2. Materials: The toxicology folder containing the provisional report, chain of custody, toxicology request form, chromatograms and instrument reports and supporting documentation (e.g. notes to file, correspondence, etc.) and a computer with internet access and barcode reader.

2.19.3. Protocol:

2.19.3.1. Review the toxicology request form and verify the following have been properly documented on the provisional report. (Both the probable cause of death (COD) and pertinent history must be complete prior to certification. Contact the medical examiner or pathologist for missing information if needed. Submit the case to the evidence technician or the forensic document specialist for correction if needed.)

2.19.3.1.1. Decedent name
2.19.3.1.2. Date of case receipt
2.19.3.1.3. Date of specimen collection
2.19.3.1.4. Specimen type
2.19.3.1.5. Collection site
2.19.3.1.6. Submitter
2.19.3.1.7. Specimen condition

2.19.4. Review the chain of custody (COC) and verify that there is an entry for every test or procedure conducted on the case and that documentation for each entry (e.g. chromatogram, note to file, etc.) is present. Check the accuracy of the COC with regards to aliquot amount and transaction purpose. Make sure the duplicate amount matches the total aliquot. Submit the case to the appropriate forensic chemist for correction if needed.

2.19.5. Review the testing data and verify that the results have met the criteria for reporting and the results are properly documented on the provisional report. Verify that screening data is consistent with confirmation data. Submit the case for additional testing or corrections if needed.

2.19.6. Review the toxicology results with regards to the case information provided on the toxicology request form. Submit the case for additional testing if needed.

2.19.7. Certify the report by logging into MEIS and selecting the toxicology administration menu item and then toxicology approval menu items. Using the barcode reader, scan the report into the system. Verify report approval by noting the return of the toxicology folder number at the top of the screen.

3. Evidence Handling and Storage

3.1. Biological Evidence

3.1.1. The proper selection, collection, submission and storage of biological specimens for toxicological analysis are important if the analytical results are to be accurate and their subsequent interpretations scientifically sound. Details are provided in TOX-P13 Toxicology Specimen Requirements.

3.1.1.1. Ideally, a minimum of 10 mL (20 mL preferable) of blood, serum, or plasma should be submitted for cases requiring comprehensive toxicological analyses. If less than 10 mL sample is submitted, the toxicologist shall identify and prioritize the analyses in order to maximize the value of the toxicological analyses. If less than 5 mL of sample is submitted, NC OCME Toxicology may not be able to complete all of the required examinations (for further details see TOX-P14 Test Assignment).

3.1.1.2. Blood samples should be collected in 20 mL scintillation vials (or equivalent) containing 4.4 g sodium fluoride to preserve the samples. NC OCME provides 20 mL scintillation vials containing this preservative to the Medical Examiners and pathologists across the state of North Carolina through a third-party vendor.

3.1.1.3. It is recognized that hospital or clinical specimens collected pursuant to medical treatment and death may be collected in blood vials or test tubes with or without preservatives. Such exceptions shall be noted on the toxicology request form with an appropriate description of the evidence (e.g. serum, plasma, etc). See SOP 001.

3.1.1.4. In general, antemortem specimens with the chronologically earliest collection times should be analyzed whenever possible. Urine can be used as a screening tool in order to conserve limited blood specimens.

3.1.1.5. Postmortem samples should be labeled with type (e.g. blood, bile, urine, liver) and location of blood sample collection (e.g. iliac, heart, subclavian). Failure to label postmortem evidence appropriately limits the value and interpretation of toxicological results.

3.1.1.6. A completed Toxicology Request Form (TRF) should accompany the submitted specimens.

3.1.2. At the time of receipt, the specimen label information should be inspected and compared to TRF to verify that the information matches. Any discrepancies should be documented on the TRF.

3.1.2.1. TRF should document unique specimen identifiers and the evidence should be labeled accordingly.

3.1.2.1.1. The TRF is used to document the numbers, types, and volumes of specimens along with their unique specimen identifiers (S#). The specimens are labeled with the unique S# barcode label and an identical label placed on the corresponding row of the TRF.

3.1.2.1.2. The TRFs are used to document additional information such as deficiencies in external packaging, evidence seals, and unusual types or conditions of specimens.

3.1.2.1.3. A unique Toxicology folder identifier (T#) shall be written on the TRF and a corresponding barcode label placed on a folder in which all documentation and data for the case will be placed.

3.1.3. Storage of Biological Evidence

3.1.3.1. Specimens are received by evidence receiving in the toxicology laboratory (room 2611) and should, as appropriate, be refrigerated as soon as possible to preserve their condition. (SOP 001).

3.1.3.1.1. Blood, urine, vitreous humor, and other liquid specimens shall be stored at 2-8ºC.

3.1.3.1.1.1. Blood received in purple topped test tubes deemed for genetic testing shall be stored at -15 ºC immediately following accessioning.

3.1.3.1.2. Tissue samples (e.g. liver, brain, spleen) shall be stored frozen at approximately -15ºC.

3.1.3.2. Whenever evidence is not actively being aliquoted/analyzed, it shall be stored in evidence refrigerators/freezers. Access to the refrigerators is limited to toxicology personnel.

3.1.3.3. Biological evidence in the process of examination generally will not remain in short term storage for longer than 180 days.

3.1.3.4. Upon completion of a case, OCME samples are retained in the freezer for up to two years (space permitting).

3.1.3.5. Specimens are discarded by batch COC procedure which includes verification of final location, specimen identification and witness of discard.

3.1.3.6. Aliquots of case specimens may be sent to an outside laboratory for testing at the behest of a toxicologist.

3.1.3.7. Case specimens may be sent for outside testing (at requester's expense) or retained for an additional three years with appropriate request (Outside testing request letter) from next-of-kin or a court order. (TOX-P16)

3.2. **Prescription Products and Other Evidence**

3.2.1. The Toxicology laboratory will often receive case evidence in addition to biological specimens. Details for proper handling, storage, and testing of such evidence are outlined in SOP-001, TOX-P19, and SOP-105.

4. **Sampling Procedure**

4.1. Sampling evidence is critical in toxicological analysis. One must be sure that what is sampled is truly representative of the total sample submitted. The analyst must take into consideration the level of homogeneity among submitted biological specimens. In order to perform a toxicological analysis, a representative sample (aliquot) shall be removed from the biological specimen. Refer to specific assay SOP for specimen aliquot specifications.

4.1.1. Biological fluids should be gently shaken and/or inverted several times to ensure sample homogeneity prior to removal of an aliquot for analysis.

4.1.2. Due to the viscous nature of many biological fluids, an aliquot should be drawn into and expelled from the pipette tip a minimum of three times prior to dispensing the aliquot for extraction and/or analysis. This allows the inner surface of the pipette tip to be thoroughly coated allowing for a more accurate dispensed volume.

4.1.3. As post mortem specimens tend to be in various stages of decomposition and clotting, not all are suitable for aliquot using a micropipette. If the sample aliquot does not flow smoothly into the pipette tip or the volume of specimen is too small for a full aliquot, the sample must be aliquoted into a test tube placed on a precision balance so that the aliquot can be accurately weighed and recorded.

4.1.3.1. If the weighed aliquot is greater than ±5% of the aliquot amount used for calibration, an appropriate sample multiplier shall be assigned. (See SOP calculations section).

4.1.4. Biological fluids may be diluted prior to analysis for a number of reasons (e.g. small sample volume or analyte concentration is greater than Upper Limit of Linearity (ULOL)).

4.1.5. For screening purposes, no less than one half of the SOP required specimen volume should be sampled unless instructed otherwise by a senior chemist or toxicologist.

4.1.6. For quantitation and confirmation analyses that require dilution due to the analyte concentration exceeding the Upper Limit of Quantification (ULOQ), a minimum of 50µL aliquot should be taken. If a smaller aliquot is required (e.g. 10-40µL), a second aliquot ≥50µL shall be included for comparison.

4.1.7. Occasionally, a biological specimen or piece of physical evidence may contain a target analyte at such high concentration that a serial dilution is necessary to accurately measure it.

4.1.7.1. Plan the dilution in as few steps as possible to get the estimated concentration of the unknown into the target concentration range (calibration curve). Have a colleague check the math.

4.1.7.2. If the initial aliquot in a serial dilution is weighed, such as clotted blood, liver homogenate, or gastric contents, then all subsequent aliquots shall be weighed to maintain consistency.

4.1.7.3. In order to report the final concentration of the unknown:

4.1.7.3.1. The "on-column" amount (Pre-multiplier) of the unknown must fall within the calibration curve range.

4.1.7.3.2. The max/min of the calculated concentrations of the duplicate analysis shall not exceed 25% (see SOP calculations section).

4.1.7.4. The sample multiplier is determined by dividing the standard aliquot volume (the volume in which the calibrator calculations are based) by the actual volume of aliquot taken for that assay.

4.1.7.5. Routine dilution of matrix is accomplished through the aliquoting of reduced specimen volume; no blank matrix is added unless otherwise specified in the assay specific SOP.

4.1.8. Tissues (liver, brain, spleen, etc) are generally considered to be homogeneous throughout the submitted tissue - therefore a portion of the tissue may be sampled and analyzed (SOP 005).

4.1.8.1. Weigh a specific quantity of tissue (usually 4 grams but other amounts may be used if necessary but water should be adjusted accordingly). Add appropriate volume of water to obtain final dilution (e.g. add Di water to 4.0g tissue for a total weight of 16.0g and a final dilution of 1:4). Homogenize sample with a tissue homogenizer.

4.1.8.2. Analyze appropriate mass of tissue homogenate as described in specific method SOP.

4.1.8.3. Multiply the analyte concentration by dilution factor and report tissue concentration in mg/Kg, ng/g, or mg%.

4.1.9. Gastric contents may contain pills, pill fragments and/or partially digested food material and therefore are NOT homogeneous samples (SOP 105). For total gastric concentration utilize the following procedure.

4.1.9.1. Prior to the analysis of gastric contents, weigh the total gastric contents. Record total weight.

4.1.9.2. Homogenize the gastric contents prior to sampling to ensure homogeneity. If the addition of water is required to adjust for viscosity, record the amount added (g). Calculate dilution factor: Final weight (gastric + water) / sample weight (gastric) = dilution factor

4.1.9.3. Weigh the appropriate aliquot amount according to SOP. Consult case history to determine if a dilution is required.

4.1.9.4. Multiply the analyte concentration by the total gastric content weight and report gastric contents results as mg of analyte. Use the suffix 'Total" to indicate that the amount of analyte is the total mg detected in the gastric.

5. **Engineering Control to Prevent Specimen Switching (Test Tube Label and Transfer Procedure)**

5.1. This procedure is to be followed for all specimen extractions and preparations, with the exception of Volatiles (SOP 101), to prevent the occurrence of switched samples.

5.2. Prior to retrieving specimens from storage, a 1-2-inch length of colored masking tape should be pre-labeled with the identity of each sample to be extracted/prepared on the worklist, including one for each standard and control.

5.2.1. The accession numbers should be copied from the worklist. It is acceptable to use only the significant digits of the accession number (e.g. S140010268 may be recorded as 10268) and to distinguish between duplicate aliquots of the same sample with a "d" and/or the dilution factor e.g.:

5.2.1.1. std 1
5.2.1.2. std 2
5.2.1.3. BLK
5.2.1.4. 5835
5.2.1.5. 5835d
5.2.1.6. 9225
5.2.1.7. 9225 1:10
5.2.1.8. QC

5.3. Handling one sample at a time, a piece of pre-labeled tape should be affixed to an aliquot tube and the number upon it compared to the corresponding standard mixture or vial from which the aliquot is to be taken.

5.3.1. If the numbers do not correspond, either the incorrect samplevial was chosen or the specimen identity was incorrectly written.

5.3.2. This is a check that the correct sample is not only retrieved from storage but that it is being aliquoted into the correct specimen tube and ultimately analyzed under the correct accession number.

5.4. Once all the standards, controls, and samples have been aliquoted, the extraction/preparation steps are followed.

5.4.1. At each step where the specimens are transferred to a clean test tube, handling only one sample at a time, the tape is peeled from the old tube and placed on the new tube and hence follows the sample transfer.

5.5. Labeling of autosampler (ALS) vials should be performed using a permanent marker and from the worklist (not specimen containers or test tube labels).

5.6. Final transfer of the extract to its corresponding ALS vial should also be performed by handling one sample at a time.

5.7. The identity of the sample, read from the original tape on the extraction tube, should be compared to the permanent marker identification written on the ALS vial.

5.8. Any discrepancy will result in the samples concerned being repeated.

5.9. The analyst will annotate this in a Note-to-File and enter the samples concerned into the LIMS and record it on the results summary sheet after data reduction.

5.10. Failure to follow the above procedure will result in disciplinary action.

6. **Procedures to Prevent Specimen Contamination/Cross-Contamination**

6.1. The following procedures shall be followed to prevent inadvertent contamination of and/or mixing of specimens (cross-contamination).

6.1.1. Glassware:

6.1.1.1. Volumetric flasks and other glassware used primarily for the preparation of analytical standards shall be segregated from glassware used to contain and dilute case specimens.

6.1.1.2. Following use, glassware used to prepare analytical standards and reagents shall be thoroughly rinsed with appropriate solvent (SOP-010).

6.1.1.3. Following use, glassware used to prepare analytical standards and reagents containing biological fluids or tissues shall be first rinsed with 10% bleach solution and then cleaned using a glassware cleaning brush and warm soapy water followed by a Di water rinse. If the glassware is too small to be cleaned by this method, the following procedure should be followed:

6.1.1.3.1. Rinse with 10% bleach solution followed by tap water.

6.1.1.3.2. Rinse with 2N Sulfuric acid solution (SOP-102) followed by tap water.

6.1.1.3.3. Rinse with ~50% Ammonium hydroxide in water solution followed by tap water.

6.1.1.3.4. Rinse thoroughly with Di water followed by methanol.

6.1.2. Specimen containers shall be handled one at a time.

6.1.3. During sampling procedure, disposable pipette tips shall be discarded and replaced after the aliquot of each specimen is completed.

6.1.4. Pasteur pipettes shall be discarded after each use. Do not use the same Pasteur pipette to transfer more than one reagent or specimen.

6.1.5. The outside of pipettes shall be cleaned (using Kim-wipe(s) and 10% bleach solution) whenever visibly soiled and after specimen aliquoting is complete.

6.1.6. When visibly soiled, PPE (gloves, face shield, lab coat) shall be immediately removed and replaced with clean PPE.

6.1.7. Bench paper shall be discarded upon completion of extraction (replaced sooner if visibly soiled) and work area wiped down with 10% bleach solution.

7. Laboratory Quality Control

7.1. Reagents

7.1.1. Chemicals used in the preparation of reagents for use in qualitative and quantitative analyses should be of at least ACS (American Chemical Society) grade or better.

7.1.2. Solvents used in the toxicology laboratory shall be high quality, low residue solvents (e.g. HPLC grade, Omnisolv, Optima etc.).

7.1.3. Water used in reagent preparation should be either deionized (certified LCMS grade for LCMS reagents) or Millipore Systems or reverse osmosis – for all others (abbreviated throughout this manual as Di water).

7.1.4. The following information shall be recorded on the bottle/container for all purchased reagents upon receipt/opening:

7.1.4.1. Date of receipt (important for chemicals with limited shelf life)
7.1.4.2. Date opened
7.1.4.3. The initials of the person opening the bottle
7.1.4.4. The expiration date (or “no expiration”) as appropriate.

7.1.5. Reagents, chemicals and supplies shall be handled, transported, stored and used in a manner that maintains their quality at an acceptable level. In general, the manufacturer's recommendations for storage conditions as specified on the product label should be followed.

7.1.6. All prepared reagents shall be documented and maintained in the *Reagent Preparation Log Book* (unless described as "one time use" or "make fresh daily in SOP). A different final volume of reagent not listed in a particular reagent preparation instruction (SOP) may be made as long as volume adjusted amounts of constituents are used and the final volume prepared is documented in the reagent preparation logbook.

7.1.6.1. Reagents prepared for one-time or daily use shall have their ingredients' quantities and lot numbers listed on the standard & control work sheet or other batch specific document.

7.1.7. When preparing a new reagent, the following information is to be documented in the *Reagent Preparation Log Book*:

7.1.7.1. Unique lot#
7.1.7.2. Reagent name
7.1.7.3. Reagent concentration
7.1.7.4. List of constituents, their volumes and lot numbers
7.1.7.5. Date made
7.1.7.6. Preparers initials
7.1.7.7. Date-of-first-use
7.1.7.8. SOP(s) for which the reagent is to be used

7.1.8. In the toxicology laboratory, the reagent QC check is typically performed within a batch of samples as evidenced by the acceptable performance of the calibrators and controls with the particular reagent. As such, the documentation of the QC check is typically found in the raw data of the load that corresponds with the reagent's date-of-first-use. Analysts are required to notify the QA chemist when a new reagent exhibits a QA problem on its designated QC check batch. Documentation of the problem and resolution are required.

7.1.9. All laboratory prepared reagents/solutions will be clearly labeled to include at a minimum:

7.1.9.1. Reagent identity
7.1.9.2. Reagent concentration
7.1.9.3. Preparer's initials
7.1.9.4. Date of preparation
7.1.9.5. Lot number
7.1.9.6. Affiliated SOP
7.1.9.7. National Fire Protection Association (NFPA) Hazard Diamond

7.1.10. In general, all solutions and reagents (unless otherwise indicated in a specific SOP) may be stored at room temperature for up to 1 year after preparation date or when the solution/reagents fails to perform as expected .

7.1.11. All chemicals and commercial reagents should be replaced when their stated expiration date or shelf life has expired and/or when they fail to perform as expected.

7.1.12.

Reagent Type (examples)	**Expiration Date (From day made/opened)**
Solvents (MeOH, Di water, Acetone, Acetonitrile…)	Manufacturer defined
Acidic Solutions (1M Acetic Acid, 2N H_2SO_4)	6 Months
Buffers (4.5pH acetate Buffer)	6 Months
Ammonium Hydroxide	2 Months
Fisher pH 5 solution (Acid/Neutrals)	1 Year
Specialty Reagents (e.g.Trinders)	See Individual SOP's

7.2. **Preparation of Blank Blood**

7.2.1. Obtain human packed blood cells from a blood bank (e.g. UNC Hospitals).

7.2.2. Weigh approximately 2.2g of sodium chloride and 17.5g of sodium fluoride on a top-loading balance and place in a 2000mL Erlenmeyer flask.

7.2.3. Dilute with 500 mL Di water and mix until dissolved.

7.2.4. Cut the top off of the blood bank blood bag and pour the contents into the 2000 mL Erlenmeyer flask with the salt solution. Swirl until mixed

7.2.5. Pour the mixture into a screw-cap reagent jar labeled or empty 4L Di water bottle with date prepared, preparer's initials, and "Blank Blood".

7.2.6. Each lot of blank blood will be given a unique lot number; "BB" followed by the date prepared in mmddyy format. If two or more lots are prepared on the same day, the subsequent lot number(s) will represent the date of the following working day(s)

7.2.7. Analyze each lot of blank blood using the following assays:

7.2.7.1. LC screen
7.2.7.2. Organic base screen
7.2.7.3. Acid/neutral screen
7.2.7.4. Salicylates screen
7.2.7.5. Volatiles screen

7.2.8. The approving Forensic Scientist or Toxicologist shall clearly note on the data, the bottle, and post in the laboratory any analyte for which the lot is positive.

7.2.9. A lot of blank blood that has tested positive for an analyte of interest shall not be used for associated assays.

7.2.10. Records from blank blood preparation and screening shall be scanned and saved in pdf format and maintained in the laboratory for a minimum of five years.
7.2.10.1.1.1. S:\toxicology\QAQC\QA-QC Standards\Blank Blood

7.2.11. Store at 2-8°C for up to one year.

7.3. **Preparation of Blank Liver**

7.3.1. Approximately 1 lb of food grade bovine liver is used in the preparation of drug free liver homogenate.

7.3.1.1. Weigh liver on a top loading balance and record weight.

7.3.1.2. Measure out a volume of Di water (mL) that is three times greater than the total mass (g) of the liver (giving a desired 1:4 dilution (tissue: total volume)).

7.3.1.3. Cut liver into small pieces (≤1/2 inch). Place about 1/10th of the liver into a blenderwith enough Di water to cover.

7.3.1.4. Blend the mixture until completely homogenized and pour contents into a clean and empty 4 liter Di water container.

7.3.1.5. Repeat steps 2 and 3until all the liver has been homogenized and the total volume of Di water added.

7.3.1.6. Swirl contents of the 4L container to mix well. Label with "LH (date prepared in mmddyy format)", analyst's initials, and date prepared.

7.3.2. Drug free liver homogenate is not screened for the presence of drugs prior to use, but is tested on a load by load basis with routine casework.

7.3.3. Store at -20°C for up to five years.

7.4. Blank Urine

7.4.1. Drug free urine is obtained from volunteers employed in the toxicology laboratory.

7.4.2. Drug free urine is not screened for the presence of drugs prior to use, but is tested on a load by load basis with routine casework.

7.4.3. The blank urine is only acceptable as a negative control if all analytes of interest are shown to be absent during analysis.

7.5. Standard Reference Materials

7.5.1. Standard reference materials shall be at least of United States Pharmacopeia – National Formulary (USP-NF) quality and are used to prepare calibrators or controls.

7.5.2. Whenever possible, standard reference materials should be of analytical quality and include the supplier's Certificate of Analysis (COA) to document traceability, purity, accuracy, precision and homogeneity.

7.5.3. Patented standard reference materials may be obtained directly from the pharmaceutical manufacturer.

7.5.4. In the absence of a supplier's COA, standard reference materials must be verified and documented prior to use. Verification should include full spectrum GCMS analysis with comparison to library spectra and the absence of additional/interfering chromatographic peaks; or the use of other analytical techniques as necessary (HPLC/DAD, LCMS or UV/VIS spectrophotometry) to generate suitable definitive instrumental data.

7.5.4.1. Whenever possible, non-certified standard reference material should be analytically compared to a certified standard (acceptance range is within 15% diff.).

7.5.5. Verification data should be labeled with analyst's initials and date.

7.5.6. Verification data should be stored electronically; the data is scanned and a PDF copy created. The data is to be saved into an appropriately named folder in the following location: S:\toxicology\QAQC\QA-QC Standards**"Drug Name"**

7.5.7. If COA's are available for both Calibrator and QC material, verification may be performed within a batch of samples when the standard is used as a calibrator or control. As such, the documentation of verification may be stored with the unique load number that contains the batch analysis raw data.

7.5.8. Standard reference materials shall be stored in a manner that maintains their quality. In general, powders are stored at room temperature (unless otherwise stated on COA), aqueous and methanolic solutions are stored at 2-8°C unless otherwise indicated by the supplier or in a specific method SOP.

7.5.9. Allow all standard reference materials and reagents to come to room temperature prior to starting procedures.

7.5.10. All measurements reported in or supporting examination documentation must be recorded in such a manner that the results are traceable to standard reference materials (See Section 7.6).

7.6. Records

7.6.1. The *Drug Inventory Logs* will be used to track the storage locations of purchased drug reference materials.

7.6.1.1. Certified Drug Standards (Ampules)

7.6.1.2. Stock Drug Standards (Room 2603)

7.6.1.3. DEA controlled (Safe)

7.6.2. The *Drug Standard Preparation Log* (room 2603) will be used to track the preparation of stock and working calibration standards.

7.6.3. The *Drug Control Preparation Log* (room 2603) will be used to track the preparation of stock and working positive controls.

7.6.4. The *Volatile Standard Preparation Log* (room 2601) will be used to track the preparation of stock and working standards and controls used in volatile analysis.

7.6.5. The *Reagent Preparation Log Book* (room 2603) will be used to track the preparation of reagents.

7.7. Balances

7.7.1. All analytical balances will be checked daily (prior to use) for accuracy using Class ASTM-1 weights or better. Record the weights on the monthly balance check sheet (S:\toxicology\Instrument Logs and Methods\Maintenance Logs\Balance Monthly Log.xlsx) with the date and analyst's initials.

7.7.2. Weights used to check balance accuracy shall be sent to vendor for re-certification every three years.

7.7.3. The following table lists examples of balance class types with appropriate check weights. If the individual balance does not fit into one of these categories, use three different weights within its range or as approved by the QAQC Chemist.

Balance Type	Balance Examples	Check Weights
Analytical	Mettler AB135-S	5.0 (± 0.10) mg
(0.01mg)		2000.00 (± 4.00) mg
Precision	Mettler XP603S	0.0500 (± 0.001) g
(0.001 – 0.0001g)	Mettler AG245	50.0000 (± 0.10) g
	Fisher ALF204	
Rough (0.01g)	Mettler PM300	0.10 (± .02) g
	Fisher ALF2002	50.00 (± 0.10) g

7.7.4. Accuracy must be established for daily balance checks or after a balance has been put into service after purchase, calibration, maintenance or repair. The following are guidelines for performing balance checks:

7.7.4.1. The check weights listed in the table in 7.7.3 are weighed and recorded on the monthly balance check sheet.

7.7.4.2. The accuracy of each weight should meet the criteria in 7.7.3.

7.7.4.3. If the accuracy of a weight(s) is within the acceptable range, the balance is ready to use.

7.7.4.4. If the accuracy of a weight is outside the acceptable range, ensure that the balance is level and clean prior to rechecking.

7.7.4.5. Perform balance performance check again. If the balance accuracy fails again, use the internal calibration function (if available) or external calibration to recalibrate balance. Repeat balance check. Record all pre and post calibration measurements on log sheet.

7.7.4.6. If, after the above mentioned actions, the result of the balance check is still outside of the acceptable range, re-calibrate the balance for a second time. Repeat balance check. If it again fails, the balance shall be taken out of service (and labeled as such) until maintenance and/or calibration are performed by a qualified and approved vendor.

7.7.4.7. Record the service call on the analytical balance QC sheet kept with each analytical balance.

7.7.4.8. If the balance is taken out of service for repair/maintenance, perform a full balance check prior to putting the balance back into service.

7.7.4.9. All balances are cleaned, serviced and calibrated annually by an outside vendor. Record the service call on the analytical balance QC sheet kept with

each analytical balance. Each balance shall be labeled with the date of calibration and when the next calibration is due.

7.8. pH Meter

7.8.1. Calibrate the pH meter as needed. Calibration results are saved in the pH meter's memory. Refer to the pH meter's instrument manual for these procedures.

7.8.2. Prior to each use, check instrument calibration by measuring the appropriate reference buffers and record the results on the pH Meter Calibration Log

7.8.3. The reference buffers chosen should bracket the expected pH value range of the solution, if possible.

7.8.4. The pH values must be within ± 0.3 units of the pH value stated on each individual reference buffer's labeling.

7.8.5. If the calibration values are within the accepted limits, the pH meter is ready to use for reagents.

7.8.6. If the calibration values are not within the accepted limits, re-calibrate and/or troubleshoot as necessary.

7.8.7. Rinse the electrode with Di water, after each measurement.

7.8.8. Reference buffers shall be replaced when the expiration date listed on the manufacturer label is reached.

7.8.9. Keep reference buffer bottles tightly sealed and free of contamination.

7.8.10. Do not reuse an aliquot of reference buffer or return it to the original bottle.

7.8.11. Refer to the pH meter instrument manuals for recommendations on proper use and storage, good laboratory practices, correct applications, problem samples and trouble shooting.

7.8.12. For non-critical pH measurements, an approximate pH may be determined using pH paper.

7.9. Pipettes

7.9.1. Fixed volume, variable volume, multichannel and repeater pipettes shall have their calibration evaluated and certified by an approved vendor bi-annually.

7.9.2. Repair documentation generated by the vendor is maintained in the office of the QA/QC chemist as well as electronically (S:\toxicology\QAQC\Pipette Calibration)

7.9.3. As needed, clean the outside of pipettes with 10% bleach solution.

7.9.4. If a pipette appears to be out of calibration between normally scheduled performance/calibration checks, the pipette will be labeled as “Out of Spec” and removed from the bench until the pipette can be serviced.

7.9.4.1. The “Out of Spec” pipette may still be used for non-quantitative purposes such as transferring of sample extract from a test tube to an autosampler vial.

7.9.5. All pipettes shall be uniquely identified and appropriately labeled with the date of calibration and when the next calibration is due.

7.10. **Refrigerators/Freezers**

7.10.1. In the toxicology laboratory, all refrigerators and freezers that are used to store biological evidence or critical reagents are monitored continuously to ensure the appropriate storage temperature. See Smart-Vue Policy Manual and Procedure Manual.

7.10.1.1. Temperatures are monitored using a Thermo Smart-Vue system.

7.10.1.1.1. A container of glycol based solution is placed in each unit to be monitored.

7.10.1.1.2. A temperature probe is placed into the glycol solution

7.10.1.1.3. Readings are recorded by the system every 60 minutes

7.10.1.1.4. If two consecutive readings are outside of acceptance range an alarm is triggered.

7.10.1.1.4.1. The sensor readout on Smart-Vue software changes from green to red

7.10.1.1.4.2. The system notifies both the QA/QC chemist and Laboratory Supervisor via phone, text, and email.

7.10.1.1.5. The system requires acknowledgment of each alarm as well as explanation/corrective action entries.

7.10.1.1.6. For refrigerators, the temperature should fall between 1-8°C.

7.10.1.1.7. For freezers, the temperature should fall below -15°C.

7.10.1.1.8. A review of the Smart-Vue temperature data is performed monthly by the QA/QC chemist or proxy. Documentation of the review is stored electronically in: S:\toxicology\QAQC\Temperatures

7.10.1.1.9. Any corrective action(s) taken shall be logged in the Smart-Vue software as well as an annotation added to the appropriate temperature graph.

7.10.2. If the temperature should fall just outside the acceptable range, the thermostat should be adjusted accordingly to bring the temperature back into the acceptable range.

7.10.2.1. Any adjustments to refrigerator/freezer thermostats must be reported to the QA/QC chemist and annotation made on the appropriate sensor graph in Smart-Vue.

7.10.3. For extreme temperature changes (e.g. freezer above 0°C, refrigerator below 0° C or greater than room temperature), all biological evidence and critical reagents should be removed immediately from the affected unit and placed in alternative refrigerators and/or freezers (if possible). The refrigerator or freezer should be placed out of service, labeled as such until it can be repaired and the repair should be documented on the temperature log.

7.10.4. The quality of critical reagents exposed to extreme temperatures may be compromised and the affected reagents should undergo a performance check or verification prior to their use on casework.

7.10.5. Maintain temperature logs for all refrigerators and freezers for at least five years.

7.11. Heating Blocks

7.11.1. In the toxicology laboratory, heating blocks are generally used for the evaporation or derivatization of samples. With each use, the temperature of the heat block should be checked with a thermometer to ensure the temperature falls within the recommended temperature range.

7.11.2. Adjust the thermostat as necessary to achieve the desired temperature.

7.11.3. The temperature should fall within the recommended temperature range of the assay.

7.11.4. If the correct temperature cannot be achieved, remove the heat block from service and label it as such until it can be repaired or replaced.

7.11.5. Documentation of the performance of the heat block is evidenced by the acceptable performance of the calibrators and controls with each batch analysis.

7.12. Turbo-Vap Evaporators

7.12.1. In the toxicology laboratory, Turbo-Vap evaporators are used only for the evaporation of extraction solvents. Therefore, the exact temperature of the water bath does not need to be measured by NIST traceable thermometers.

7.12.2. If the set temperature cannot be achieved, remove the evaporator from service and label it as such until it can be repaired. Document the repair in the evaporator instrument logbook.

7.12.3. The operating nitrogen flow pressure can be adjusted with the pressure control knob located on each Turbo-Vap. The operating nitrogen flow pressure shall not exceed 20psi.

7.12.4. Documentation of the performance of the evaporator is evidenced by the acceptable performance of the calibrators and controls with each batch analysis.

7.13. **Solid Phase Extraction Manifolds**

7.13.1. Solid Phase Extraction (SPE) Manifolds are located within each chemical fume hood in the wet lab (room 2601)

7.13.2. Each unit utilizes four banks of twelve nozzles to apply positive pressure (house nitrogen) to assist in forcing specimens and extraction solvents/reagents through SPE columns.

7.13.2.1. For low pressure applications (≤ 5PSI), the selector switch is turned to the left (counterclockwise).

7.13.2.2. For high pressure applications (≥ 20PSI), the selector switch is turned to the right (clockwise).

7.13.2.3. Placing the selector switch in the center position stops the nitrogen flow.

7.14. **Homogenizer (Silent Crusher M) (SOP-005)**

7.14.1. The homogenizer is used to liquefy tissue and other solid-like specimens to make them homogenous and easier to extract and analyze.

7.14.2. A portion of the tissue is weighed and combines with a known amount of water (typically at a 1:4 ratio (tissue: total volume)).

7.14.3. The homogenizer uses a stainless steel generator to pulverize and homogenize the tissue sample.

7.14.4. To prevent cross-contamination of other specimens, the generator is to be disassembled and thoroughly cleaned after each use.

7.14.4.1. After use, remove generator from the homogenizer motor and hold over sink.

7.14.4.2. Rinse generator thoroughly with 10% bleach solution.

7.14.4.3. Place homogenizer under running water to rinse off bleach and any excess tissue.

7.14.4.4. Carefully disassemble generator and clean each part thoroughly, using 10% bleach, running water, forceps, cotton swabs, and/or kim-wipes to ensure cleanliness.

7.14.4.5. Place the disassembled generator parts on a dry paper towel to dry until next use.

7.15. **Antistatic Electrode**

7.15.1. The antistatic electrode is a “U” shaped device for use with the analytical balance located in room 2603.

7.15.2. When powered on, the electrodes emit a stream of electrons that deionizes anything that passes between them.

7.15.3. The electrode should be powered off following use.

7.16. **Centrifuges**

7.16.1. All recommended centrifuge speeds are approximate in order to achieve the appropriate separation of layers. Therefore, no intermediate tachometer checks to verify the speed of the rotor are required.

7.16.2. Clean centrifuge as needed.

7.16.3. If a centrifuge is taken off line for repair/maintenance, it should be labeled as “Out of Service” until it is repaired. Documentation of the repair is maintained in the office of the laboratory supervisor.

7.17. **Thermometers**

7.17.1. Thermometers used in the toxicology laboratory are used to measure non-critical temperatures (target range of 5 - 10 deg C). Therefore, NIST certified/traceable thermometers are not required.

7.18. Incubators (Ovens)

7.18.1. Incubators (ovens) are currently not in use in the Toxicology Laboratory.

7.19. Fume Hoods and Biological Safety Cabinets

7.19.1. Fume Hoods and Biological Safety Cabinets are certified annually.

7.19.2. For proper operation refer to the Office of the Chief Medical Examiner Safety Manual

7.20. **UV/VIS Spectrophotometer**

7.20.1. On each day of use the analyst will:

7.20.1.1. Turn on the instrument and visually verify that the automatic initialization operation passes (Smiley Face displayed on instrument screen).

7.20.1.2. Perform a “self-test” on the instrument and print a copy of the test results for the batch. Save the “self-test” to the instrument computer. See SOP 075 or the instrument manual for detailed instructions.

7.20.2. Every two years, have the instrument manufacturer perform a certified performance test (PT) on the instrument. This PT includes the following:

7.20.2.1. Program File Integrity Test

7.20.2.2. Wavelength setting test

7.20.2.3. Security Mode test

7.20.2.4. Wavelength Accuracy Test (using NIST certified Hg lamp)

7.20.2.5. Photometric Accuracy Test (using NIST traceable photometric accuracy filters)

7.20.2.6. Audit Trail Test

7.20.3. Maintain PT documentation in the instrument log.

7.21. **Gas Chromatographs**

7.21.1. Most toxicology procedures are performed in batch and therefore most maintenance procedures are performed prior to running a batch of samples. Record all maintenance in the Instrument Log with date and initials.

7.21.2. Day-of-Use

7.21.2.1. For quadrupole GCMS systems, perform a tune. A hardcopy copy of the Autotune shall be maintained for at least five years. Autotune results shall meet the following criteria: SOP-060.

7.21.2.2. Run a Performance Check (e.g., a specific instrument test mix or a batch calibrator). A hardcopy shall be turned in with each associated load.

7.21.3. As Needed

7.21.3.1. The following should be replaced as needed: (SOP-061)

7.21.3.1.1. Septa

7.21.3.1.2. Liner
7.21.3.1.3. Gold seal
7.21.3.1.4. Syringe
7.21.3.1.5. Check and replace gas cylinders
7.21.3.1.6. Clip front portion of column and reinstall

7.21.3.2. Condition column

7.21.3.2.1. Short method example: Hold oven temperature 10 degrees above Final Temperature for 15 minutes

7.21.3.2.2. Long method example: Hold oven temperature to the column's maximum temperature for 600 minutes (overnight)

7.21.3.3. Detector maintenance

7.21.3.4. Clean MSD source

7.21.3.5. Replace NPD bead (SOP-062)

7.21.3.6. Replace NPD ceramics

7.21.3.7. Clean FID

7.21.3.8. Bake out ECD

7.21.3.9. Replace or clean jet

7.21.4. Daily

7.21.4.1. Bake out Headspace autosampler oven and transfer line. (SOP-101)

7.21.5. Quarterly

7.21.5.1. Inspect gas filters and replace as needed.

7.21.6. Biannually

7.21.6.1. Replace GC/MS Columns

7.21.6.2. Computer maintenance

7.21.6.2.1. Archive methods, macros and data files onto long term storage media.

7.21.6.2.2. Once archived, data files and sequence files more than one month old may be deleted from the hard drive.

7.21.7. Annually

7.21.7.1. Replace GC/NPD and GC/FID column.

7.21.7.2. Schedule an on-site preventative maintenance service call as per the service agreement.

7.21.7.3. After the instrument has been shut down or significant maintenance has been performed, verify that the instrument is fit for use by running a test-mix solution, positive control or calibrator to ensure appropriate sensitivity, chromatography and separation of the components of the mixture.

7.21.7.4. Retain instrument verification documentation in the instrument logbook.

7.22. **LC/MS Systems**

7.22.1. Record all maintenance and problems on the Monthly Maintenance Worksheet. (SOP-071)

7.22.1.1. Record all problems and troubleshooting in the instrument log book

7.22.2. Ensure that all gradient and wash solvents are full

7.22.3. Routine maintenance schedule (or as needed, refer to Monthly Maintenance Worksheets):

7.22.3.1. Replace column monthly

7.22.3.2. Clean transfer ion tube and sweep cone monthly

7.22.3.3. Clean tube lens, skimmer, and Q00 quarterly

7.22.3.4. Calibrate bi-annually

7.22.4. Annually

7.22.4.1. Schedule an on-site preventative maintenance service call as per the service agreement.

7.22.4.2. Computer Maintenance

7.22.4.2.1. Data from instrument hard drives (C:) drives shall be backed up to the share drive (S:) and deleted from the local hard drive.

7.23. **Hydrogen generator**

7.23.1. Add Di water weekly or as needed.

7.23.2. Change moisture filter as needed.

7.23.3. Change deionizer bags every six months.

7.24. **Milli-Pore Deionized Water Systems**

7.24.1. Vender performs preventative maintenance biannually or as needed

7.25. **New Instrument Installation**

7.25.1. Obtain documentation from the instrument service representative that demonstrates that the instrument performs to the manufacturer's specification.

7.25.2. Load methods, macros and libraries and test their functionality.

7.25.3. Perform self-check or autotune (GCMS), as needed.

7.25.4. After methods have been loaded or created, run check solutions, positive controls or calibrators to demonstrate the instrument is fit for use (e.g. appropriate sensitivity, specificity, accuracy, precision, chromatography or identification of the components of the mixture).

7.25.5. Retain instrument verification documentation in the instrument logbook.

7.25.6. A summary of the verification shall be sent to the Laboratory Manager for approval prior to placing the new instrument into service.

7.25.7. If the instrument does not meet expectations or acceptance criteria, label it as "not in service" and notify the Laboratory Manager as soon as possible.

8. **Method Validation**

8.1. **Introduction**

8.1.1. Validation is a set of experiments that establish the effectiveness and reliability of a method or changes to a method. The goal of validation is to establish evidence that a method is capable of performing at the level of its intended use, and to identify the method's limitations.

8.2. **When to validate methods**

8.2.1. Development of a new analytical method

8.2.2. Changes made to an existing analytical method

8.2.2.1. Addition of new analyte
8.2.2.2. Change in analytical column type.
8.2.2.3. Change in mobile phase.
8.2.2.4. Change in mobile phase gradient/temperature program.

8.2.2.5. Change in ion collection (MS).
8.2.2.6. Major changes in extraction procedure

8.2.2.6.1. Different solvent used
8.2.2.6.2. Change from e.g. liquid/liquid to solid phase extraction (SPE).
8.2.2.6.3. Change in e.g. SPE manufacturer.

8.2.3. To demonstrate consistency between an established method/instrument and a new method/instrument.

8.3. Method Validation Parameters

8.3.1. Qualitative analysis (Qual):

8.3.1.1. Ion suppression/enhancement (LC/MS)
8.3.1.2. Precision (at decision concentration only)
8.3.1.3. Interference studies
8.3.1.4. Carryover
8.3.1.5. Limit of Detection

8.3.2. Quantitative analysis (Quant):

8.3.2.1. Ion suppression/enhancement (LC/MS)
8.3.2.2. Calibration model
8.3.2.3. Interference studies
8.3.2.4. Limit of Quantitation
8.3.2.5. Limit of Detection
8.3.2.6. Bias
8.3.2.7. Precision
8.3.2.8. Carryover
8.3.2.9. Extract stability

8.4. Requirements for conducting method validation experiments

8.4.1. Ion suppression/enhancement – Post Extraction Addition (Qual/Quant) (Only necessary if target analyte and corresponding internal standard have retention times greater than 0.05 min. apart).

8.4.1.1. Two different sets of samples are prepared and the analyte peak areas are compared to evaluate the matrix effects.

8.4.1.2. The first set (set 1) consists of neat standards, analyte(s) and internal standard(s), at both low and high concentrations.

8.4.1.2.1. Each neat shall be injected three times.

8.4.1.3. The second set (set 2) consists of unspiked extracted samples (a set for each matrix – minimum 3 of each matrix type).

8.4.1.3.1. After extraction, each pair is fortified with analyte(s) - at low and high concentrations - and internal standard.

8.4.1.3.2. Each sample is then injected three times for a minimum of nine injections of each matrix at each concentration.

8.4.1.3.2.1. Multiple specimens at each level may need to be extracted if sample volume is an issue.

8.4.1.4. From this data, the average peak areas of each set are calculated.

8.4.1.5. The percent ion suppression or enhancement is then calculated for each concentration:

$$\textit{Ion suppression or enhancement} = \left[\left(\frac{\textit{average area of set 2}}{\textit{average area of set 1}}\right) - 1\right] \times 100$$

8.4.1.5.1. Ion suppression or enhancement, for each matrix, at each concentration, shall be less than 15%.

8.4.2. Recovery

8.4.2.1. Recovery experiments are not required but may be performed along with Ion suppression/enhancement.

8.4.2.2. Two different sets of samples are prepared and the analyte peak areas are compared to evaluate the matrix effects.

8.4.2.3. The first set (set 1) consists of extracted samples containing analyte(s) and internal standard(s) at both low and high concentrations (minimum 3 of each matrix type).

8.4.2.3.1. Each specimen shall be injected three times.

8.4.2.4. The second set (set 2) consists of unspiked extracted samples (a set for each matrix – minimum 3 of each matrix type)

8.4.2.4.1. After extraction, each is fortified with analyte(s) - at low and high concentrations - and internal standard.

8.4.2.4.2. Each sample is then injected three times for a minimum of nine injections of each matrix at each concentration.

8.4.2.5. The percent recovery is then calculated for each concentration:

$$\left(\frac{\textit{average area of set 1}}{}\right.$$

$$\textit{Percent recovery} = \frac{\text{average area of set 2}}{\text{average area of set 2}} \times 100$$

8.4.3. Calibration model (Quant):

8.4.3.1. Standard curve fitting is determined by applying the simplest model that adequately describes the analyte concentration/internal standard response ratio using appropriate weighting and statistical tests for goodness of fit.

8.4.3.2. Five sets of matrix specific blanks and six-level (minimum) calibration curves shall be extracted.

8.4.3.2.1. The levels of the calibration curve should be evenly spaced.

8.4.3.2.2. The range of the calibration curve should encompass the working calibration range of the assay.

8.4.3.2.3. Each set is injected at least once to allow for a minimum of six replicates of each calibration level and blank.

8.4.3.2.3.1. The six sets can be extracted and analyzed on the same day or over multiple days.

8.4.3.3. The combined data of the six replicates shall be used to establish a calibration model.

8.4.3.3.1. The origin shall be ignored in calibration models.

8.4.3.3.2. Compare effectiveness of different weighting schemes at reducing method error by calculating the sum of the relative error (ΣRE) for each calibration point.

8.4.3.3.3. Use the least amount of weighting that minimizes error to a satisfactory level.

8.4.3.4. Once the calibration model is established, it should not be altered to achieve acceptable results for a given analytical run.

8.4.4. Interference studies (Qual/Quant):

8.4.4.1.1. Endogenous compounds - A blank sample (ten for each matrix type) with no internal standard added shall be extracted and analyzed to ensure the analyte(s) of interest is/are not detected.

8.4.4.1.2. Internal Standard - A blank sample with internal standard added shall be extracted and analyzed to ensure the analyte(s) of interest is/are not detected.

8.4.4.1.2.1. Only necessary when a matching deuterated internal standard is used.

8.4.4.1.3. Target analyte(s) - A blank sample with a high concentration of target analyte(s) (equal to or greater than the highest calibrator) and no internal standard added shall be extracted and analyzed to ensure the analyte(s) of interest are not contributing to the internal standard.

8.4.4.1.3.1. Only necessary when a matching deuterated internal standard is used.

8.4.4.1.4. Commonly occurring non-target analytes - A blank sample with a high concentration of non-target analyte(s) (e.g. organic base screen standard 1, standard 2, and/or standard 3, opiate QC, cocaine QC, Benzodiazepine QC, etc.) and no internal standard added shall be extracted and analyzed to ensure the analyte(s) of interest and internal standard are not detected.

8.4.5. Limit of Quantitation (LOQ) (Quant)

8.4.5.1. The LOQ shall be the concentration of the lowest standard of the calibration curve that can be measured with acceptable accuracy and precision.

8.4.5.2. The “low concentration” specimens used in the accuracy and precision experiments, and meet all acceptance criteria, can be used in the determination of an acceptable LOQ for the assay (See sections 8.4.7 and 8.4.8).

8.4.6. Limit of detection (LOD) (Qual/Quant)

8.4.6.1. LOD estimates using the Calibration Curve:

8.4.6.1.1. For methods that follow a linear calibration model

8.4.6.1.2. Minimum of three calibration curves constructed across working range

8.4.6.1.3. LOD estimate from standard deviation of *y*-intercept (σ_y) and the average slope (m_{avg}) as:

$$LOD = (3.3\sigma_y) / m_{avg}$$

8.4.6.2. Half lowest calibrator:

8.4.6.2.1. A matrix specific sample (three for each matrix type) shall be fortified with internal standard and a concentration of target analyte(s) that is one half that of the lowest calibrator (Quant) or control (Screen).

8.4.6.2.2. The samples shall be analyzed in triplicate and the process repeated over three separate days.

8.4.6.2.3. LOD peak criteria:

8.4.6.2.3.1. Peak must have a signal to noise ratio of 250 or higher (as calculated in data processing software.

$$\textit{Signal-to-noise ratio} = \frac{\textit{Height of analyte}}{\textit{Amplitude of noise}}$$

8.4.6.2.3.2. Peak must elute at the expected retention time (2.12.1.1)

8.4.6.2.3.3. Peak shape must be symmetrical (Gaussian) in shape.

8.4.6.2.3.4. Ion ratio confirmation must meet pre-defined method criteria.

8.4.6.2.4. If the above criteria are not met, the concentration of the lowest calibrator that meets the above criteria shall become the limit of detection.

8.4.7. Bias (Quant)

8.4.7.1. Bias calculations shall be conducted over a period of five days and can be performed concurrently with precision studies.

8.4.7.2. Each day, a calibration curve and two concentrations of test specimens (low and high) shall be extracted.

8.4.7.3. The test specimens shall be analyzed in triplicate and the concentrations calculated against that day's calibration curve.

8.4.7.4. The within- and between-run bias shall be calculated using the following formulas:

$$\textit{Within-Run Bias (\%)} = \frac{\textit{Mean calc. value for day \# – expected value}}{\textit{Expected value}} = X\ 100$$

$$\textit{Between-Run Bias (\%)} = \frac{\textit{Mean calc. value for all days – expected value}}{\textit{Expected value}} = X\ 100$$

8.4.7.5. For common biological fluids, within-run and between–run accuracies should be below 20% at each concentration level. (Ethanol accuracies shall be below 10%.)

8.4.8. Precision

8.4.8.1. Precision calculations shall be conducted over a period of five days and can be performed concurrently with accuracy studies.

8.4.8.2. Each day, a calibration curve and two concentrations of test specimens (low and high) shall be extracted.

8.4.8.3. The test specimens shall be analyzed in triplicate and the concentrations calculated against that day's calibration curve.

8.4.8.4. The within-run and intermediate precision shall be calculated using the following formulas:

$$\textit{Within-Run CV (\%)} = \frac{\textit{SD of day \# samples}}{\textit{Mean calc. value for day \#}} \; X\,100$$

$$\textit{Intermediate CV (\%)} = \frac{\textit{SD of combined means for each level}}{\textit{Grand mean for each level}} \; X\,100$$

8.4.8.5. For common biological fluids, within-run and intermediate precision shall be below 20% at each concentration level. (Ethanol precision shall be below 10%.)

8.4.9. Carryover (Qual/Quant)

8.4.9.1. To be performed for assays with no administratively established carryover policy in place.

8.4.9.2. Multiple blank specimens (no internal standard), a calibration curve, and sets of two specimens fortified with high concentrations of target analytes are to be extracted.

8.4.9.3. Each fortified specimen is to be injected followed by a blank.

8.4.9.4. The highest concentration to show no analyte detected in the subsequent blank (above the LOD) shall become the carryover limit.

8.4.9.5. The carryover limit shall be verified by triplicate injections of that concentration each followed by a blank.

8.4.10. Extract stability

8.4.10.1. It is not always possible to analyze specimens immediately following extraction. It is therefore important to determine the stability of extracts, refrigerated or at room temperature for extended periods of time.

8.4.10.1.1. Extracted specimens (such as described in section 8.4.3) shall be stored to simulate the conditions of the instrument on which they are analyzed.

8.4.10.1.2. The specimens shall be reanalyzed on day 3 and day 7 to determine the degree of degradation, if any, over time.

8.4.10.1.2.1. Peak areas of target analytes and internal standards shall not vary greater than ± 20%.

8.4.10.1.2.2. If specimen degradation is evident after 3 days, further studies shall be performed to determine the stability time interval by analyzing the specimens daily.

8.4.11. Documentation

8.4.11.1. All aspects of the validation plan shall be documented with the following:

8.4.11.1.1. Description of all parameters validated

8.4.11.1.1.1. If any parameters were not validated, a reason must be documented

8.4.11.1.2. Matrices analyzed
8.4.11.1.3. Concentrations of validation samples
8.4.11.1.4. Raw data (or location of raw data storage)
8.4.11.1.5. Analysts involved
8.4.11.1.6. Instrumentation
8.4.11.1.7. Instrumentation methods
8.4.11.1.8. Dates
8.4.11.1.9. Results and calculations
8.4.11.1.10. Conclusions

8.4.11.2. All documentation pertaining to the validation of a method shall be retained for a period of 10 years after the method has been taken out of service.