MNPD Crime Laboratory

Toxicology Technical Procedures Manual

Metropolitan Government of Nashville & Davidson County
Police Department

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1 Introduction

1.1 Scope

Toxicology is the study of how alcohol, drugs, and poisons affect the human body. Forensic Toxicology is defined as the application of toxicology for the purposes of law. This discipline involves the use of various scientific procedures and instrumentation for the purpose of identifying ethanol andother volatiles in blood and possibly other biological fluids. The results of those analyses are compiled into an official report that can be used in a court of law.

Types of cases analyzed can include, but are not limited to: DUI/DUID, vehicular manslaughter, etc. Due to the diversity in sample matrix, sample condition, and the number of drugs available, the Toxicology Unit has established routine guidelines for sample analysis. These procedures allow the individual scientist the flexibility to choose the best validated methods to use in performing their analysis; however, acceptable screening and confirmatory practices must be followed.

1.2 Continuing Education

Employees of the Toxicology Unit should attend continuing education training, preferably on an annual basis. These trainings will allow employees to maintain networks with other toxicology labs and keep up with advancements in analytical technology, drug trends, testing methods, new instrumentation, and reference resources. These opportunities are provided by obtaining approval to attend any of the following, though this list is not exhaustible:

Society of Forensic Toxicologists (SOFT)

Southern Association of Forensic Scientists (SAFS)

American Academy of Forensic Sciences (AAFS)

ANSI-ASQ National Accreditation Board (ANAB)

American Society for Mass Spectrometry (ASMS)

Borkenstein Courses on Alcohol and Drugs

Vendor Training (i.e. Agilent, ABSciex, etc.)

Requests for training will be completed by the Toxicology Unit Supervisor or designee and will then be submitted to the Crime Lab Business Manager and Lab Director or their designees for approval of time and funds to attend the training.

On an annual basis, the Toxicology Unit Supervisor will identify and discuss training needs for their staff. The conversation regarding training needs can occur through email, direct conversation, or in a unit meeting. The anticipated training is documented in the yearly budget spreadsheet. The Toxicology Unit Supervisor or designee will also evaluate the effectiveness of

training received. Evaluation is documented in a quality management system (QMS)workflow at minimum.

1.3 Reference Literature

The Toxicology Unit will provide access to forensic reference materials. These can include journal articles, spectral libraries, reference publications and other literature dealing with alcohol and drug analysis. All forensic scientists are encouraged to review new literature in an effort to remain up to date with new techniques and developments in these disciplines.

The Toxicology Unit Supervisor or designee should review journals and publications received in the laboratory. Appropriate publications and copies of applicable journal articles should be distributed to all members of the section for their review. Reviews will be documented in the "Literature Review Log" workflow inthe QMS.

1.4 Toxicology Analytical Workflow

- The following is the general analytical workflow for routine toxicological analysis. The DUI/DUID protocol is designed to identify alcohol and volatilesthat can impair driving using one level of testing: alcohol analysis (I).

  - DUI/DUID Cases

    ↓

    Level I

    BAC/Volatile Determination

    ↓

    ↓

    Issue Report

- Additional testing may be conducted if requested by the investigating officer or District Attorney's office. Additional testing can also be completed at the discretion of the Toxicology Supervisor, Quality Manager, Lab Director or trained analyst with management approval. If a test is requested that the laboratory does not have the capability or procedure for performing, the case can be sent to an outside reference laboratory for analysis.

  The toxicology unit will determine the best methods to be used during specimen analysis. For example, if an officer only suspects drugs in a case with low sample volume then alcohol analysis might not be performed as a priority.

2 Evidence Handling and Storage

2.1 Evidence Submission

The proper selection, collection, submission and storage of biological specimens for toxicological analysis are important in providing the customer with the most accurate analytical results and scientific interpretation, preventing contamination and deterioration, and providing sample security.

- A minimum of 10mL of blood should be submitted for toxicology cases requiring comprehensive analysis. If less than 10mL of sample is submitted the MNPD-CL may not be able to perform all of the requested examinations. In this situation, analysis shall be prioritized to maximize the value of toxicological testing.

- Blood samples should be collected in gray top tubes containing potassium oxalate and sodium fluoride to preserve the sample. MNPD-CL provides gray top tubes containing these preservatives to the customers in the form of manufactured BAC kits.

- There could be situations in which specimens are collected in blood vials without the previously mentioned preservatives (e.g. a car accident in which an individual needs medical treatment and blood is drawn in a hospital setting). Such exceptions shall be noted in the case file with a description/photo of the tube/container.

- A collection tube for blood that is not consistent with the gray top tube does not indicate that the sample is not suitable for testing or accurate analysis. However, the notation in the case file is necessary in the event that the integrity of the sample comes into question.

- The time of collection of each specimen will be recorded in the case file.

- In general, the specimens with the earliest collection times should be analyzed whenever possible.

2.2 Storage of Toxicology Biological Evidence

- Specimens received in the Toxicology Unit shall be refrigerated to prevent deterioration of the specimen. It is acceptable for specimens to be left at room temperature temporarily because of the preservative in the collection tubes. However, refrigeration is the preferred method of storage.

- Whenever evidence is not actively being analyzed it shall be stored in evidence refrigerators assigned to the forensic scientist. Access to the Toxicology Unit refrigerators is limited to toxicology personnel and upper management.

- Upon completion of a toxicology case, the evidence is retained in an evidence refrigerator for at least 1 year. The submitting agency can request that the evidence be held longer and the reason for holding the case shall be documented in the case file.

2.3 DUI Evidence Handling

The following guidelines apply to the receiving, accessioning, processing, storage, and destruction of toxicology samples in the MNPD-CL.

2.3.1 Evidence Receipt

- Toxicological evidence is routinely submitted to the MNPD-CL via the MNPD Evidence Storage Division (ESD) with the MNPD form 110 which indicates a request for service. The BAC kits are transported to the MNPD-CL Evidence Receiving Unit (ERU) in response to the request for service.

2.3.2 Evidence Accessioning

- Toxicology evidence is accessioned in the toxicology prep room within the Toxicology Unit. Refer to Toxicology Laboratory Procedures Manual "Case Login Procedure".

- Each BAC kit is accessioned individually. Evidence must be itemized and labeled appropriately before opening another kit to prevent mislabeling of evidence.

- The BAC kit seal integrity shall be checked. Any visible lack of seal or tampering shall be documented in the case file and on the report if the evidence could have been compromised. The BAC kit condition shall be visually documented by uploading an image into the LIMS system (ex. picture taken with a camera, scan, etc.) If there is a problem creating visual documentation, then the kit condition must be noted in the case file.

- The BAC kit is opened and the blood vials and Alcohol/Toxicology request form removed. The evidence is inspected and any deviation from the normal condition is documented in the case file (ex. vials leaked, vials not provided by MNPD-CL, etc.)

- The forensic scientist will fill out the laboratory portion of the request form and log in the information received from the submitting agency in the LIMS system (ex. subjects information, specimens received, agency information, chain of custody, analysis requested, case notes, etc.) In the event that information is already populated in the case, the forensic scientist shall verify the accuracy of the information in the case file.

- The forensic scientist will itemize the evidence within the case file, give a description of the evidence, and generate a barcode in LIMS. The LIMS system will generate a barcode with a specific identification number upon completion of the case entry. The evidence is labeled with the barcodes and a visual documentation of the alcohol/toxicology request form and evidence received is created. The visual documentation can be in the form of a photograph or scan that is saved in the case file.

- The BAC kit container can be discarded once the items are itemized and de-containerized. The kit container is scanned to a "destroyed" location.

- Once accessioned, the assigned scientist will take possession of the evidence by scanning the evidence to themselves or a storage location. The chain of custody will be kept using LIMS.

- The forensic scientist assigned to the evidence will store the evidence in an assigned refrigerator when evidence is not actively being analyzed.

- Once the forensic scientist takes possession of the evidence, it is their responsibility to verify that the lab number/information on the evidence is consistent with the information on the Alcohol/Toxicology request form.

2.3.3 Evidence Processing and Storage

- The toxicology unit currently performs alcohol analysis on every case. The forensic scientist assigned to the case usually completes all analysis on the case from start to finish, but this is not a requirement. In cases other than routine DUI (ex. rush case) the evidence workflow can vary based on type of request.

- Evidence in the process of being worked is scanned out of a storage location into the possession of the scientist. Evidence should not be left unattended in the laboratory for extended periods of time and must be scanned back into a storage location at the end of the work day. Evidence can be left in the possession of the scientist for short periods of time (ex. bathroom, lunch breaks, etc.)

- Blood tubes shall remain sealed with a cap when not actively being sampled.

- Once alcohol testing is complete, a report is generated and the evidence is stored in an assigned refrigerator or long term storage until disposal. Evidence assigned to long term storage is placed in racks and the tube position recorded in LIMS.

2.3.4 Requests for Independent Laboratory Testing

The MNPD-CL has the ability to send evidence to an independent laboratory. There are situations that could require the Toxicology Unit to have external testing performed. Some examples are, but not limited to, the following:

- The MNPD-CL Toxicology Unit does not have the capability of performing a specific test requested by the customer.

- The customer requests that an independent laboratory perform the analysis. This requires approval from upper management.

- The MNPD-CL toxicology unit can use an independent lab during new method development to verify results.

- An independent lab can be used in the event that a toxicology forensic scientist personally knows the subject involved in a MNPD-CL case to prevent a conflict of interest situation.

- The attorneys in a case can request that a sample be re-analyzed by an independent laboratory. In this situation, a court order is required to release the sample to another laboratory. The MNPD crime lab is not responsible for vetting other laboratories in this instance.

- In the event that the customer makes the request for independent testing, the reason for the request must be documented in the case file.

- The chain of custody in JusticeTrax will track the evidence or aliquot of the evidence that was sent to an independent laboratory.

- If a report is received in the MNPD-CL by the independent lab then it will be scanned into a record in the case file. It is the responsibility of the crime lab to provide the report to the requesting customer.

2.3.5 Destroying Evidence

- The evidence will be sealed and stored in a long term storage location for at least 1 year after the report is issued. After that time if no motions, court orders, or request to hold evidence have been received on a particular case, the evidence will be destroyed.

- The samples will be scanned in the LIMS system as “destroyed” and the chain of custody will reflect the date and time of this disposition.

- The evidence will be placed in biohazard trash for destruction by the laboratory’s waste provider.

3 Sampling Procedure

- The sampling of evidence is a critical component in toxicological analysis. The purpose of a good sampling technique is to ensure that what is being sampled is a true representation of the sample as a whole. The analyst must take into consideration several factors to ensure a homogenous aliquot of biological matrix is analyzed.

3.1 Homogeneity

- Biological fluids should be placed on a rocker or manually inverted several times to ensure sample homogeneity prior to removal of an aliquot for analysis.

- In the event that rocking or inverting the biological sample does not allow for a homogenous mixture, then the sample can be vortexed to allow for more thorough mixing.

3.2 Dilutions

- Biological fluids may be diluted prior to analysis for several reasons (e.g. small sample volume or analyte concentration greater than the upper limit of quantitation (ULOQ)).

- Biological fluids shall be diluted in an appropriate blank matrix such as blank blood or as otherwise indicated in a specific method SOP.

- For screening purposes, no less than ½ of the SOP required specimen volume shall be sampled.

- Quantitation and confirmations that exceed the ULOQ require a minimum amount of biological fluid to accurately perform a dilution analysis. This minimum volume should be determined during method development and stated in the SOP for the specific method.

3.3 Freeze/Thaw and Refrigeration

- It is common for calibrators and controls prepared in a biological matrix to be frozen or refrigerated for stability purposes. The biological matrix should be completely thawed and thoroughly mixed to ensure homogeneity before aliquots are sampled for analysis. Calibrators, controls, internal standard, blank blood, samples, reagents, etc. stored in the refrigerator/freezer should be allowed to get to room temperature before analysis.

3.4 Pipetting Technique

- Pipetting technique is important to achieve accurate and reproducible results. The following are procedures that the toxicology unit shall follow to ensure consistent aliquoting of standards, solvents, specimens, etc.:
  - Verify that the pipette being used is within the calibration range and that the calibration is up to date.

  - Pre-wet the pipette tip: Aspirate and expel an amount of liquid several times before aspirating for delivery. Failure to pre-wet can cause significantly lower delivery volumes.

  - Allow liquids to equilibrate to ambient temperature prior to pipetting. Working at a constant temperature minimizes variation of pipetted volume.

  - Examine the pipette tip before and after dispensing sample.

  - Use standard (or forward) mode pipetting: depress the plunger to the first stop, immerse the tip into the liquid and aspirate by releasing the plunger slowly. Remove the pipette from the liquid and depress the plunger to the second stop to dispense the entire contents. This mode of pipetting yields the best accuracy and precision.

  - Draw the sample up slowly into the pipette tip. Liquid continues to flow into the tip for a short time after the plunger stops.

- When aspirating, hold the pipette vertically and pull the pipette straight out from the center of the container. Holding the pipette at an angle as it is removed from the liquid alters the volume aspirated. Touching the sides of the container causes wicking and loss of volume.

- Ensure the correct pipette tip is used while pipetting. Mismatched tips and pipettes can result in inaccuracy, imprecision, or both.

- When pipetting, a consistent plunger pressure and speed shall be used to ensure repeatable results.

4 Toxicology Quality Procedures

Summary

The following toxicology quality procedures apply to the analysis of toxicology results. Specific method procedures may specify different criteria. Refer to the Quality Manual for instructions on deviations from validated test methods/procedures. A deviation will be documented through a workflow in the QMS.

4.1 Batch Analysis

In order to maximize efficiency within the Toxicology Unit specimens are routinely grouped into batches (e.g. a batch of blood alcohols or a batch of blood extractions).

- When performing a batch analysis, only one blood tube shall be open at a time and then sealed before opening another blood tube. This is to prevent cross-contamination of evidence.

- The work area should be cleaned with bleach or Clorox wipes after sampling evidence to prevent contamination and the presence of blood borne pathogens.

- Each batch analysis must contain a sufficient number of controls to monitor the performance of the assay. This number is dependent on the size of the batch and the type of analysis.

- For batch analysis that utilizes an instrument autosampler, the position of each vial in the autosampler must be verified by a different analyst against the sequence list. This vial verification is documented with initials and date on the sequence list.

- If a batch fails to meet the QA/QC acceptance criteria, documentation of specimen aliquots and the reason for batch failure will be included in each case file or related calibration package (See sections 4.3.1 through 4.3.5).

4.2 Quality Assurance and Quality Control Definitions:

Linear Range: For most chromatographic assays, The Limit of Quantitation (LOQ) and Upper Limit of Quantitation (ULOQ) are defined in terms of the concentration of the lowest and highest calibrators used in the calibration curve.

Limit of Detection (LOD): The LOD is the lowest concentration where an analyte can be confirmed as present, but not necessarily quantitatively accurate.

Limit of Quantitation (LOQ): The lowest concentration at which quantitative results can be reported with a high degree of confidence. To include the LOQ in a calibration response curve, the LOQ response must be at least three times greater than the negative control response, should have acceptable ion ratios and should back calculate within 30% of the target concentration.

Upper Limit of Quantitation (ULOQ): The ULOQ is the highest calibrator concentration included in the calibration response curve with acceptable ion ratios and the back-calculated concentration should fall within 20% of the target concentration.

Calibrators: Solutions that are prepared from a standard reference material purchased from an ISO certified supplier that are used to calibrate an assay. If possible, the calibrators should be prepared in a matrix similar to that of the specimens.

Internal Standard (IS): An analyte or analytes that are added in a consistent amount to samples, controls, and calibration standards. The internal standard can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. Ideally, the internal standard should be structurally or pharmacologically similar to the analyte being tested.

Calibration Curve: A series of known analytes and levels used in determining the concentration of an analyte in an unknown sample by comparing the unknown to a set of standard calibrators of known concentration.

Controls: A set of known standards (negative or positive) that are run against a calibration curve to verify that the calibration curve is valid and that the instrument is functioning properly. Passing controls indicate to the analyst that the results obtained for samples analyzed with the controls are acceptable.

4.2.1 Qualitative Assays

- A minimum of one negative and one positive control/reference standard are required to be run along with the samples in each run.

- Mass spectral qualitative results can be reported with a retention time match and a response above a cutoff control

4.2.2 Control Criteria:

- In quantitative analysis, controls may be purchased or prepared in-house. In-house controls should be prepared from a different manufacturer or different lot of standard material than used in calibrator preparation. If this is not possible, the same manufacturer and lot numbers of standards may be used for both calibrators and controls when made separately and by different analysts.

- Control values obtained with a batch analysis will be entered in a spreadsheet for monitoring by the Toxicology Supervisor or designee.

4.2.3 Mass Spectral Quality Control

- Full Scan Mass Spectral Identification: It is the responsibility of the experienced analyst to interpret mass spectra based match criteria to identify an analyte. No rigid match criteria are defined to prevent misidentification or under-identification of an analyte. The experienced analyst will base identification on several factors, such as retention time, unique ions, ion abundance, signal to noise, literature references, and library probability based matching scores. The identification of an analyte should be based on a spectral library match or comparison to an actual standard reference material. This standard reference material is required to verify the mass spectrum and retention time.

- Qualitative Identification: The analyte shall be compared to a positive control or calibrator containing standard reference material and a negative control. The case sample analyte peak should be well resolved, the retention time should match the standard and the mass spectra should contain all the major ions unique to the analyte. A poor spectral match with missing ions or additional ions indicates a weak signal, background noise or co-eluting substance and should be reviewed by the Toxicology Supervisor.

4.2.4 Re-injection Criteria and Documentation:

There may be situations in which calibrators, controls, and case samples need to be re-injected. Common reasons for reinjection include the following:

- Poor analyte or internal standard recovery. The sample is re-injected to determine whether poor recovery was injection related or extraction related.

- Poor chromatography or interference.

- There is a physical instrumentation issue (e.g. bad column, bubble in the lines, leaking column) that requires the sample to be re-injected.

- The calibrator/control/sample exhibits possible carryover. This may occur when the autosampler needle/syringe is not properly rinsed after injection from a previous sample or the presence of an extremely high analyte concentration that is not flushed out of the system before the next injection. If carryover is suspected, a negative control or blank should be run and proven negative and then the sample re-injected. If the analyte in question is still present, the sample could be contaminated and reanalysis shall be performed. Any sample run directly after a sample which has an analyte concentration greater than the highest calibrator and is positive for the same analyte should be re-injected to assess carryover.

- Re-injected samples shall be identified as such in the sequence run or data and the original sample injection shall not be overridden so comparison of the two injections is possible.

- Document on the original chromatogram that the original injection was unacceptable and the reason for the reinjection.

4.2.5 Re-extraction Criteria and Documentation:

There may be situations in which calibrators, controls, and case samples need to be re-extracted and/or reanalyzed. Common reasons for re-extraction include, but are not limited to, the following:

- The sample exhibited poor chromatography or poor recovery and reinjection did not improve the chromatography or response.

- The sample overloads the column and a dilution is necessary to achieve good chromatography and accurate measurement.

- The sample evaporated, so there is not enough sample left for re-injection.

- The sample was contaminated by a previously injected sample.

The reason for the re-extraction shall be documented in the case file and the original and re-extracted data must be included in the case file.

4.3 Validation of Methods

The goal of validation is to demonstrate that a method is successfully performing as intended, produces reproducible results, and identifies limitations. Methods shall be validated when it is necessary to verify the method's performance. Examples include:

- The implementation of a new analytical method

- Modifications to a method to improve performance

- Demonstrate equivalence between an established method/instrument and a new method/instrument.

The parameters to be evaluated depend on the circumstances in which the method is to be used. SWGTOX guidelines should be followed when performing a new method validation. A validation plan providing direction as to what criteria will be analyzed, the timeframe of the validation, and the participants will be established prior to new method validation. The following are guidelines for the criteria that should be evaluated for different analytical methods:

- Screening (Immunoassay)
  - Limit of Detection
  - Precision (at the decision point)
  - Dilution Integrity (if applicable)
  - Stability (if applicable)

- Screening (Other: ex. LCMS)
  - Carryover
  - Interference studies
  - Ionization suppression/enhancement (LCMS)
  - Limit of Detection
  - Dilution Integrity (if applicable)
  - Stability (if applicable)

- Quantitative Analysis
  - Bias
  - Calibration model
  - Carryover
  - Interference studies
  - Ionization suppression/enhancement (LCMS)
  - Limit of detection
  - Limit of Quantitation
  - Precision
  - Dilution Integrity (if applicable)
  - Stability (if applicable)

The original instrument validations are stored in binders within the unit and in the Toxicology Unit folder. Any new method validations or instrument comparisons will be stored electronically in the Toxicology Unit Folder or in the QMS.For specific criteria refer to the SWGTOX Standard Practices for Method Validation in Forensic Toxicology

- When a validated method is transferred to another instrument that is the same instrument type as the one used in the initial validation, the method parameters must be compared to verify the transfer was successful. The instrument must also be performance checked to verify the method is performing as expected before case samples are analyzed on that

instrument. Old methods are archived in a folder so only the current version of the method is accessible to the analysts.

- A method can be modified without undergoing a completely new validation; however, an evaluation must be conducted to confirm that the changes do not have an adverse effect on the method's performance. The following are some examples of when a method could be modified and not completely re-validated:

  - Changing the vendor for a column, but not the column type, size, or chemistry.
  - Updating an energy in the method to optimize a compound.
  - Utilizing a different deuterated internal standard.
  - Using a different transition ion to optimize a compound.

4.4 Control of Data

- The instrumentation utilized for case analysis has computer systems provided by the instrument manufacturer or the MNPD IT department. These computers have unique login criteria and each analyst has a unique personal login. These computers are located in a secure area with badge access only. If a vendor has to remotely login to the computer for troubleshooting an analyst must grant remote access, be available the entire session for monitoring purposes, and verify the vendor is not able to access the computer upon completion of the session.

- Data files and methods for each instrument are backed up on either a secure network drive (G: drive) or external hard drives. This will allow access to the raw data in the event of a hard drive issue. PDF's of calibration information are located on the secure L: drive and individual case records are imaged in LIMS.

- In the event that a new version of instrument software or a patch to the current software becomes available, the vendor can install the new version on the instrument or guide a scientist/IT manager on how to perform the installation. The new software version must be installed on all applicable instrumentation and tested by running quality control samples before being used in casework.

5 Alcohols/Volatiles by Headspace GC/FID-MS

5.1 Scope

An aliquot of each biological specimen is pipetted into a glass autosampler vial and then diluted with an internal standard solution. The vial is sealed and placed into the headspace auto sampler on the instrument for analysis. Positive cases are confirmed in a second run, negative cases can be reported after a single analysis. The concentration of ethanol or other volatiles in a dilute aqueous biological sample is directly proportional to the concentration of these compounds in the gas phase (headspace). A portion of the resultant headspace vapor above the liquid is

automatically injected into a dual column gas chromatograph (GC) equipped with a flame ionization detector (FID) and a mass spectrometer detector (MSD). Ethanol, methanol, acetone and isopropanol are each identified by retention time and mass spectrum. The concentrations of these volatile compounds are calculated automatically by the software based on linear regression of the calibration curve. In addition, 1,1-Difluoroethane is identified by retention time and mass spectrum. This compound is qualitative only and confirmed with the use of a standard and library match.

5.2 Specimen requirements

- 100 µL of blood per analysis

5.3 Reagents and Standards

- NIST traceable multicomponent alcohol kit for use as calibrators including ethanol, methanol, isopropanol, and acetone at the following concentrations: 500µg/mL, 1000µg/mL, and 4000µg/mL. Stored at 3-13^0 C.
- NIST traceable ethanol standards for use as calibrators at the following concentrations: 10mg/dL and 200mg/dL. Stored at 3-13^0 C.
- NIST traceable multicomponent alcohol mixes for use as controls including ethanol, methanol, isopropanol, and acetone at the following concentrations: 500µg/mL, 1000µg/mL, and 4000µg/mL. Stored at 3-13^0 C.
- UTAK 80 mg/dL whole blood ethanol control. Stored at (-10) - (-20)^{0}C. Controls are thawed and stored at 3-13^0 C for up to one month.
- Absolute ethanol (200 proof)
- Methanol
- Isopropanol
- Acetone
- n-Propanol
- 1,1-Difluoroethane
- Blank blood

5.4 Calibrators, Controls, and Internal Standards

5.4.1 Calibrators

Calibrators may be purchased as NIST traceable standards or prepared in-house from NIST traceable reference materials.

- Purchased Calibrators (Cerilliant)
  - 10 mg/dL and 200 mg/dL ethanol standards.
  - 500 µg/mL, 1000 µg/mL, and 4000 µg/mL multicomponent alcohol kit including ethanol, methanol, acetone, and isopropanol.
- Preparation of In-house Calibrators
  - 0.01% Ethanol Calibrator:

    Pipette 12.7µL of absolute ethanol into a 100 mL volumetric flask containing deionized water and dilute to mark. Mix by inversion. Store at 3-13° C for up to one year.

  - 0.05% ethanol and mixed volatile calibrator:

    Pipette 63 µL of absolute ethanol, acetone, isopropanol, and methanol into a 100ml volumetric flask containing deionized water and dilute to mark. Mix by inversion. Store at 3-13^0 C for up to one year.

  - 0.1% ethanol and mixed volatile calibrator:

    Pipette 126 µL of absolute ethanol, acetone, isopropanol, and methanol into a 100 ml volumetric flask containing deionized water and dilute to mark. Mix by inversion. Store at 3-13^0 C for up to one year.

  - 0.2% ethanol calibrator:

    Pipette 253µL of absolute ethanol into a 100ml volumetric flask containing deionized water and dilute to mark. Mix by inversion. Store at 3-13^0 C for up to one year.

  - 0.4% ethanol and mixed volatile calibrator:

    Pipette 504 µL of absolute ethanol, acetone, isopropanol, and methanol into a 100mL volumetric flask containing deionized water and dilute to mark. Mix by inversion. Store at 3-13^0 C for up to one year.

5.4.2 Controls

Positive controls must be purchased or prepared from a separate source as the calibrators. If In-house calibrators are utilized, controls must be NIST traceable.

- Purchased Controls

  - 500 µg/mL, 1000 µg/mL, and 4000 µg/mL multicomponent alcohol mix including ethanol, methanol, acetone, and isopropanol (Cerilliant).
  - UTAK 80 mg/dL whole blood ethanol control.
  - Negative control prepared from whole bovine blood.

- Preparation of In-house Controls:

  - See calculations for in-house calibrators for directions

5.4.3 Internal Standard

- 0.05% (v/v) n-propanol internal standard solution:

  - Pipette 1 mL n-propanol into a 2 L volumetric flask and dilute to mark with deionized water. Mix by inversion. Store at ambient temperature or 3-13^0 C for up to one year. The amount of internal standard prepared can be adjusted (ex. 500 µL n-propanol into 1 L volumetric flask).

5.5 Equipment

- Gas chromatograph with data system, FID, MSD, and a headspace autosampler.
- Columns: Restek Rtx-BAC Plus 1 (MSD) and Rtx-BAC Plus 2 (FID) capillary columns or equivalent.
- Glass headspace vials (22 mL) with PTFE-Coated Silicone rubber septa or equivalent.
- Vial seal crimper.
- Pipettes and tips.

5.6 Procedure

See Toxicology Laboratory Procedures Manual "Blood Alcohol Procedure"

5.7 Headspace Analysis

5.7.1 Gas Chromatograph Operational Parameters:

Oven	45^{0}C Isothermal
Injector	200^{0}C
Detector (FID)	300^{0}C
Hydrogen Flow	30ml/min
Air Flow	400ml/min
Make-up Flow (N2)	25ml/min

Carrier gas (He)	1.6 ml/min
➢ Inlet	
Split	
Split Ratio	30:1
Split Flow	81 ml/min
Temperature	200^{0}C
Septum purge	3ml/min
Gas Saver Mode	On

5.7.2 Headspace Sampler Operational Parameters:

➢ Sample Oven	85^{0}C
➢ Sample Valve	90^{0}C
➢ Transfer Line	100^{0}C
➢ GC Cycle	6.5 min
➢ Vial Equilibrium	15.0 min
➢ Injection Duration	0.5 min
➢ Vial Shaking	71 shakes/min

5.7.3 Mass Spectrometer Operational Parameters:

➢ MS Source	230^{0}C
➢ MS Quad	150^{0}C
➢ Solvent Delay	1.0 min
➢ EM Setting	Gain Factor (1)
➢ Acquisition Type	Scan
➢ Start Mass	20
➢ End Mass	150
➢ Threshold	150
➢ Scan Speed	1.562 (N=2) u/s
➢ Frequency	9.4 scans/sec
➢ Cycle Time	105.87 ms
➢ Step Size	0.1 m/z
➢ Flow	2.7 ml/min (Constant)
➢ Pressure	7.9 psi

5.7.4 Daily Calibration:

Calibrators are run with each day's batch sample analysis. A blank, calibrators, negative control, positive controls, and UTAK 80 mg/dL whole blood ethanol control are analyzed as part of the calibration. If the calibrators and/or controls do not meet the quality control criteria, reanalyze the calibrators and/or controls by pipetting a new calibration set. If the calibrators and/or controls still do not meet the criteria, then appropriate measures must be taken to correct the problem (instrument maintenance, open or prepare new calibrators or controls, etc.). Document the action taken in the instrument log. The calibration is valid for a work day; therefore, a confirmation batch can be setup after the initial batch in the same day. The analyst must use their

own calibration curve and the confirmation batch sequence shall begin with an acceptable negative control and UTAK 80 mg/dL control.

5.7.5 Batch Sample Analysis:

Headspace alcohol analysis is performed as a batch analysis. Analyze one UTAK 80 mg/dL ethanol control after every 10 case sample injections.

5.7.6 Vial verification/Sequence check:

The position of each vial in the headspace sampler is verified by an independent analyst and is documented by initials and date on the printed sequence table before the vials are removed.

5.8 Calculations

- Volatiles are identified based on retention times compared to calibrators on one column and by mass spectra and library match on the other column. Quantitation is calculated based on the FID calibration curve data and additional identification is from the mass spectrometer data utilizing Masshunter Data Analysis software. If an analyte shifts retention time, then the internal standard should shift the same amount for positive identification (relative retention time).

- Concentration is calculated automatically by the software based on linear regression of the 5 point calibration curve for ethanol and a 3 point calibration curve for methanol, acetone, and isopropanol based on peak response. Only 1 curve point may be dropped to improve linear regression for ethanol. The data from the Rtx-BAC Plus 2 column or equivalent is utilized for quantitative results. The data from the Rtx-BAC Plus 1 column or equivalent is utilized for qualitative confirmation results.

5.9 Quality Control

5.9.1 Daily Calibration:

Acceptable tolerance for ethanol calibrators is ±10% of the target concentration or 0.005 g%, whichever is greater. Acceptable tolerance for methanol, acetone, and isopropanol is ±10% of the target concentration or 0.005 g%, whichever is greater. Samples must be analyzed against an acceptable calibration curve.

5.9.2 Negative Control:

The negative control is injected immediately after the 0.40 g% calibrator to check for carry-over. An acceptable negative control may not contain ethanol, acetone, methanol, or isopropanol greater than 0.01 g%. If unacceptable, prepare a fresh negative control. Re-inject the 0.40 g% calibrator followed by the new negative control. If ethanol, acetone, methanol, or isopropanol is still present, perform instrument maintenance to correct the problem and document actions in the instrument log book.

5.9.3 Positive Controls:

Acceptable tolerance for the aqueous ethanol controls is ±5% of the target concentration or 0.005 g%, whichever is greater. Acceptable tolerance for methanol, acetone, and isopropanol controls is 10% of the target concentration or 0.005 g%, whichever is greater. Acceptable tolerance for the UTAK whole blood control is 10% of the target concentration.

- When a new lot of control is prepared In-house or purchased from an external source, the new control is analyzed against NIST traceable calibrators. The control must be within ±10% of the target concentration or 0.005 g%, whichever is greater.

- Case samples shall be bracketed by acceptable 0.08 controls. If one control fails, all positive case samples not bracketed between acceptable controls must be re-analyzed. If more than one control fails, all positive samples in the batch must be repeated. Negative results may be reported. Corrective actions and exceptions are documented in the instrument log book. In general, corrective actions for failed controls may include repeating the batch, recalibrating the instrument, opening new controls or making new calibrators.

- If the specified NIST traceable controls are temporarily not available (Ex. backorder), alternative concentration may be substituted (for example, substitute 0.10 g% control for 0.20 g% control) provided there is documentation, explanation, and justification in all affected case files. If there is a systemic problem obtaining external controls, the Toxicology Supervisor or Quality Manager shall be notified such that an alternative supplier can be identified and appropriate changes made to the procedure.

- Correlation of determination (r2). The r2 value for the linear regression curve must be 0.995 or greater. The correlation of determination is automatically printed on the calibration curves and tables.

- Replicate tolerance. A minimum of two analyses are required to report positive volatiles. Calculate the average and ± 5% range for the replicates. Replicates must be within the ± 5% range. Reanalyze the sample if it is outside of tolerance. The original run must be included in the case file.

- New calibrator certification:

  - NIST traceable (external) calibrators will be verified with an acceptable calibration prior to the analysis of case samples.

  - In-house calibrators: When new calibrator lots are prepared, the new calibrators are analyzed against a previously validated calibration curve.

  - The new ethanol calibrators must be within ±10% of their target concentration or 0.0050 g%, whichever is greater.

- Acetone, methanol, and isopropanol calibrators must be within ±10% of the target concentration or 0.0050 g%, whichever is greater.

- New Internal Lot Verification:

  A new lot of internal standard must be verified against a previously validated lot of internal standard. The new lot should be within ± 20% of the area of the old lot. The new lot of internal standard should be run 5 times to establish a mean response and acceptable range for case sample internal standard response.

5.10 References

- Li, Xiaohua, "Analysis of Ethanol in Blood with the Agilent 7820A GC and 7697A Headspace Sampler", Agilent Technologies Application Note. (2013).

- Firor, Roger and Meng, Chin-Kai, "Static Headspace Blood Alcohol Analysis with the G1888 Network Headspace Sampler". Agilent Technologies Application. (2004).

6 Reporting Toxicology Case Results

6.1 Ethanol/Volatiles Reporting

- A minimum of two analyses are required to report a positive ethanol/volatile. The average of the two analyses is calculated. Replicates must be within 5% of the mean. The sample must be reanalyzed if it is outside the acceptable tolerance. Negative results are reported from a single quantitative run.

- After the completion of a batch analysis, the entire run of samples to include calibrators, controls and case samples shall be reviewed by a different analyst. This can be in the form of a calibration package.

- Ethanol/Volatiles will be reported in the units (Gram %). The reported value is the average of the two replicates from the FID data truncated to three decimal places.

- Concentrations of ethanol, methanol, isopropanol, and acetone less than 0.010% Gram % shall be reported as "None Detected". Concentrations of methanol, isopropanol, and acetone less than 0.05g%, but greater than 0.01g% will be reported as "Present but less than 0.05g%".

- The upper limit of quantitation (ULOQ) for ethanol, methanol, isopropanol, and acetone is 0.40 g%. Any result greater than 0.40 g% for ethanol can be reported as "> 0.40 g%". However, the analysis can also be repeated using a 1:2 dilution. Note: Pipetting 100µL of sample into 100µL of blank blood, homogenize, then add 100 µL to vial for testing.

Any result greater than 0.4 g% for methanol, isopropanol, and acetone will be reported as "> 0.4 g%".

- All ethanol results will be reported with an Uncertainty of Measurement value rounded to the same level of significance as the reported concentration.

6.2 Qualitative Reporting

- All qualitative results shall be reported as "Present" only with no associated concentration.

- The "Presence" of an analyte indicates a concentration above a specified cutoff level.

- An analyte may be reported as "Present" only in specific situations (e.g. the analyte is not known to cause impairing effects, the presence of an analyte is not routinely analyzed by the laboratory, the quantitative procedure was performed but acceptance criteria for quantitation was not satisfied (ex. control failure) and reanalysis is not possible or practical).

- An analyte may be reported as "Present" or "None Detected" in a case with insufficient sample volume. There may be enough sample for a screen, but not enough for a quantitation extraction. A statement must be present on the report to the customer indicating that there was insufficient sample for complete analysis.

- An analyte may be reported as "Present, greater than ____" if the confirmed analyte concentration is greater than the assay ULOQ.

7 Technical and Administrative Review

- All cases shall be technically and administratively reviewed by a different analyst before a final report is issued.

- The technical reviewer shall be an individual that has been competency tested in the task that the review is encompassing.

- The review is documented in the "Technical and Administrative Review Checklist" located in the QMS. The completed form is then imaged into each individual case in JusticeTrax under case images.

- If an error is found during the review process that requires the case to be taken out of "Draft Complete", then the findings are rejected in JusticeTrax and the reason for the rejection documented followed by initials of individual making the note and the date. An email notification will be sent to the analyst and the supervisor notifying them of the rejection. The case will automatically be reverted back to "Findings Entered" status so the error can be corrected.

- The toxicology unit utilizes an alcohol/volatile calibration package to document all necessary information related to an instrument run. These calibration packages are identified by analyst's initials and date created. The calibration package/s associated with each batch of cases must also be reviewed by a different analyst before each individual case is reviewed. These calibration packages contain information related to the instrument calibration and associated quality control checks for a batch of cases. The calibration packages are located on the L:drive in the Toxicology folder under Analyst Data.

- Evidence items that are labeled incorrectly shall have the following statement on the report: "Evidence labels do not match information provided by submitting agency.".

- Evidence items that are not labeled shall have the following statement on the report: "No identifying information provided on evidence item labels"

7.1 Alcohol/Volatile Calibration Package:

- The calibration package shall include the following:

  - Instrument sequence list, including vial check.
  - Calibration curves and tables.
  - Chromatograms of all calibrators and controls.
  - Alcohol Worksheet with lot numbers of internal standard, calibrators, and controls

- Each case file shall contain case sample chromatograms and reference the appropriate calibration package information.

- There is a "Volatile Calibration Review Checklist" in the QMSthat must be completed, signed, and imaged into the calibration package to document the review.

8 Estimation of the Uncertainty of Measurement

8.1 Scope

An estimation of the uncertainty of measurement will be determined for all analytical procedures in which a quantitative measurement is reported.

8.2 Documentation

Calculations related to the reported estimation of measurement uncertainty shall be maintained or referenced in the individual case file in which measurements are made. For compounds with historical control data, the uncertainty budget spreadsheets are maintained electronically in the QMS for reference. There are unit specific spreadsheets on the L: drive for entering uncertainty of measurement alcohol control data that will be used in determining the uncertainty budget.

8.3 Uncertainty Elements

The Toxicology Unit utilizes the 8 step *Guide to the Expression of Uncertainty in Measurement* (GUM) approach in estimating measurement uncertainty.

8.3.1 Specify the measurement process

The following are the procedures that require uncertainty of measurement calculations:

- The concentration of Ethanol/Volatiles in a blood sample using the Blood Alcohol procedure with analysis by Headspace GC-FID/MS.
- The concentration of basic drugs in a blood sample with analysis by LC/MS/MS.
- The concentration of acidic drugs in a blood sample with analysis by LC/MS/MS.
- The concentration of THC and metabolites in a blood sample with analysis by LC/MS/MS.

The measurement process takes into account the concentration of the measurand, concentration of the calibrators, the instruments response for calibrators and measurand, bias, and expanded uncertainty. Each of these influences on the measuring process will have uncertainty components that will be considered.

8.3.2 Identify Uncertainty Components

There are possible sources of uncertainty that exist with any analytical procedure. The following are uncertainty sources considered in the toxicology unit procedures. These sources of uncertainty then become quantified as uncertainty components in the Measurement Uncertainty Estimation Form.

- Sampling: Homogeneity of the sample, environmental effects, sampling process, and physical state
- Sample Preparation: Extraction, Drying, Dilution
- Certified Reference Material/Controls: Uncertainty of the certified reference material and controls, ability to matrix match
- Calibration Curves: Uncertainty of reference material, preparation of the standards using volumetric flasks and pipettes, matrix matching of calibrators, instrument precision
- Analysis: Systematic errors, subjective evaluations by the analyst, environmental effects, matrix interferences, reagent purity, instrument parameters and settings, between run precision

- Data processing: Calibration model, averaging results, rounding and truncating, statistics, processing algorithms, integration parameters

Blood Alcohol Analysis Uncertainty Components

- Measurement Process Reproducibility (%CV): This component is based off of the control values ran within each batch of cases (low, mid, high controls). The control values will take into account many sources of uncertainty listed above: environmental effects, materials used, sample preparation, different analysts, instrumentation, etc. The largest % CV of the control values are considered in this calculation.

- Internal Standard Pipette : The pipettes used in the laboratory are calibrated by an external calibration lab annually. The largest % error rate at the 1000uL fixed volume for all available pipettes is used for this uncertainty component.

- Sample Pipette : A fixed pipette or dual diluter is used in the aliquoting of blood samples for this type of analysis. The largest % error for all 100uL fixed pipettes available in the lab is used for this uncertainty component.

- Certified Reference Material (CRM) for calibrators: All CRM's are being purchased from a reference material provider accredited to ISO 17034. The current provider is Cerilliant. Each CRM has an associated Certificate of Analysis used in traceability. The CRM's are not being altered prior to use in the alcohol procedure. A multi-point calibration curve is established for each alcohol run. The largest uncertainty (k=2, approximate 95% confidence interval) for any of the CRM's used in the calibration curve is used for this uncertainty component.

Drug Quantitation Uncertainty Components

- Measurement Process Reproducibility (%CV): This component is based off of the control values ran within each batch of cases (low, mid, high controls). The control values will take into account many sources of uncertainty listed above: environmental effects, materials used, sample preparation, different analysts, instrumentation, etc. The largest % CV of the control values are considered in this calculation.

- Internal Standard Pipette (Method specific): The pipettes used in the laboratory are calibrated by an external calibration lab annually. The largest % error rate for the appropriate pipette volume for all available pipettes is used for this uncertainty component.

- Sample Pipette (1000uL): A pipette is used in the aliquoting of blood samples for this type of analysis. The largest % error rate at the 1000uL volume for all available pipettes in the lab is used for this uncertainty component.

- The CRM's purchased for drug analysis (accredited to ISO 17034) are altered by dilution to create mixes used in spiking different levels for calibration curves. The sources of

uncertainty associated with calibration preparation are accounted for in the process reproducibility. This includes the tolerance of volumetric flasks and the pipettes used in the preparation of the calibrators. Each CRM has an associated Certificate of Analysis used in traceability. The largest uncertainty (k=2, approximate 95% confidence interval) for any of the CRM's that could be used in the preparation of the calibration curve is used for this uncertainty component.

8.3.3 Quantifying the Uncertainty Components

Once the sources of uncertainty have been identified and reconciled, the significance of each component must be estimated. The first step in this process is to determine what information is available for each listed source. Then the source of uncertainty is categorized as either *Type A* or *Type B*.

Random (*Type A*) Uncertainty Data

This method of evaluation of uncertainty is determined by repeating a measurement a number of times and performing statistical analysis on the results. This data can be obtained through quality control data, validation studies, collaborative studies and/or results from proficiency tests. This data is expressed as the Measurement Process Reproducibility (%CV) of controls in the Measurement Uncertainty Estimation.

- *Quality Control (QC) Data*: The QC samples may be certified reference material, purchased spiked samples, or in-house spiked matrix controls. In the Toxicology Unit the following are used as QC materials:

  - Cerilliant standards for alcohol/volatile controls
  - UTAK 0.08 for matrix matched blood controls for alcohol/volatiles
  - Cerilliant/Lipomed spiked matrix for use as controls in blood drug analysis

- The data produced by the historical quantification of QC samples provide information about the reproducibility of the analytical method. This captures the day-to-day capability of the laboratory and takes into account many variables such as: working calibration standards, various instruments, several analysts, and environmental conditions.

- Control charts are utilized to monitor trends in an analytical process. The relative standard deviation (%CV) used for uncertainty calculations shall be updated at least annually or as necessary. The following are some situations that may require an update to the calculations in addition to the annual schedule: new instrumentation, new method, addition of a new drug to the panel, etc.

- *In-House Validation Study Data*: New methods or infrequently used methods might not have an extensive amount of QC sample data generated. In this situation, validation studies that were conducted to bring the method online can be used to assist in uncertainty determinations. For example, precision and bias studies are conducted on QC samples in which the samples are analyzed several times over a period of several days, resulting in data that can be statistically analyzed.

Statistical Analysis for *Type A* Uncertainty Data

There should be enough data points acquired for uncertainty estimation to achieve a normal distribution. The data sources allow for the statistical calculation of the mean measured value of the spiked sample using the following equation:

Mean = Sum of all measured values / Number of measurements

Since the data used is part of a larger population, the standard deviation of the population is estimated using the following equation:

Standard Deviation (s) =

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}.$$

With a normal distribution of results, it can be assumed that 1 standard deviation includes 68.26% of the measurements, 2 standard deviations includes 95.45% of the measurements, and 3 standard deviations includes 99.73% of the measurements.

The Relative Standard Deviation (RSD) or % CV is estimated using the following equation:

$$\% \text{ RSD} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

The RSD or % CV is the value used to express the measurement process reproducibility in the Measurement Uncertainty Estimation Spreadsheet.

Systematic (*Type B*) Uncertainty Data

This uncertainty is derived from techniques other than repeated analysis and statistical calculations. This systematic uncertainty may be reduced by optimizing the method or measuring equipment, but can never be completely eliminated. The following are examples of *Type B* uncertainties sources that are considered in the uncertainty budget in the Toxicology Unit:

- The use of an analytical pipette to dispense sample.
- The use of a pipette to dispense internal standard.
- Uncertainty associated with Certificates of Analysis of CRM's

Systematic (*Type B*) uncertainties in the Toxicology Unit have an equal chance of being any value within the outside limits (± a), therefore a rectangular (or uniform) distribution is applied.

The standard deviation for this type of distribution is calculated using $\sigma = a/\sqrt{3}$. This distribution is applied to the pipettes used in the preparation of samples.

Type B evaluation of calibration certificates from all CRM's assume a normal distribution, a coverage factor of k = 2 and a coverage probability of 95%. Therefore, the uncertainty on the calibration certificate will be divided by 2 to arrive at the standard uncertainty.

8.3.4 Conversion to Standard Uncertainties

In this step, all calculated standard deviations are simply expressed as standard uncertainties. It is useful to ensure that common units are used throughout the budget. If the units are not the same, then all units are converted into percentages.

Ex. If measuring 10mL has a standard deviation of 0.2mL, the percentage standard deviation is calculated as 0.2mL/10mL x 100 = 2.0 %.

8.3.5 Combining Standard Uncertainties

Only uncertainties that have a significant contribution to the measurement's overall uncertainty will be considered. Values with little contribution can be removed from the estimation as long as there is data to prove the level of insignificance.

The mechanism of combining uncertainties is the Root Sum Squares technique. The combined uncertainty is equal to the square root of the sum of all uncertainty components squared.

8.3.6 Expressing Expanded Uncertainties

The laboratory's accrediting body requires that a coverage factor (k) be applied in the final step of the uncertainty measurement. The coverage factor is a number that, when multiplied by the combined standard uncertainty, produces an interval around the measurement result that is expected to include a specified percentage.

For routine measurements with a large amount of historical data:

- A (k) value of 2 represents a 95.45% confidence interval.
- A (k) value of 3 represents a 99.73% confidence interval.

- For analysis with insufficient historical data, a corrected coverage factor (kcorr) can be used based on the Student's t table.

- For example, for an analysis with no historical control data, a control is analyzed 10 times (degrees of freedom = n-1, or 9 in this example).

- Using the Student's t table, the kcorr value of 2.3 would be used to calculate the expanded uncertainty at a 95.45% confidence level.

8.3.7 Evaluate the Expanded Uncertainty

The uncertainty calculated needs to be evaluated to determine if it seems logical and reasonable for the method and meets the stated requirement. Also, the uncertainty should meet the customer's needs. For example, an expanded uncertainty of 20% for a blood alcohol is excessive but might be reasonable for a drug analysis.

If the toxicology unit determines that the expanded uncertainty is not acceptable then it might be necessary to look into why the uncertainty is high. The unit can look into method improvement, improved certified reference materials, new pipettes, etc.

8.3.8 Reporting Results with Uncertainties

- The uncertainty of measurement will be reviewed and updated at least annually or as necessary. The uncertainty of measurement may need to be re-calculated due to a significant change in the analytical process. This can include, but is not limited to, a significant change in the method, the addition of an instrument, a change in calibrators/QC, recalibration of pipettes, etc.

- The uncertainty of measurement is determined by entering numerical components into an excel spreadsheet. The accuracy of this spreadsheet is verified by the Toxicology Supervisor or designee by doing manual calculations to test the accuracy of the excel formulas.

- The toxicology unit has an administrative procedure that the ethanol replicates must fall within 5% of the average of the two aliquots. In a situation in which the reported k=3 uncertainty for ethanol falls below the guideline, a reported uncertainty of 5% will be used.

- When updating the spreadsheets containing the control values, obvious outliers can be removed in order to provide an accurate representation of uncertainty. For example, a run that included a bad injection can have the values removed to not falsely skew the data.

- The uncertainty spreadsheets are usually updated following the October schedule for pipette calibrations. Therefore, it is necessary to use the previous year's historical data and pipette calibration to determine the current uncertainty of measurement. For example, a drug request for uncertainty of measurement for a 2018 case would refer to the 2017 uncertainty data. In the event of a major procedural change, it is acceptable to use validation data and currently tracked data to determine uncertainty as long as the number of data points is deemed acceptable by the Toxicology Supervisor.

- The uncertainty of measurement for blood alcohol analysis will be stated on the final report. For drug analysis, the uncertainty of measurement will be available upon request due to the large number of drugs in the testing panel.

- When multiple measurements are performed on case specimens (ex. blood alcohol analysis performed in duplicate) the measurements shall be averaged. This will be indicated in the measurement uncertainty statement on the report.

- Since the uncertainty of measurement is only an estimate, it shall be rounded and limited to two significant figures (except for ethanol, methanol, isopropanol, and acetone which are rounded to three decimal places). The reported measurement uncertainty shall be rounded to the same level of significance (decimal places) as the reported concentration.

  - Example 1: Ethanol 0.050 ± 0.003 g%

  - Example 2: Fentanyl 15 ng/mL ± 3 ng/mL

  - Example 3: Hydrocodone 500 ng/mL ± 30 ng/mL

- Measurement uncertainty is reported at a 95.45% level of confidence for all drug analysis and at a 99.73% level of confidence for all blood alcohol analysis.

- When a request is made for a drug uncertainty of measurement a new request will be created in JusticeTrax for that case. The drugs from the original report will then be listed on a new report with the appropriate uncertainty and confidence interval. This report will undergo a technical and administrative review before being finalized and provided to the requesting party. A review checklist will be completed by the reviewer and imaged into the casefile to document this review.

8.4 References

- ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX D, Toxicology Testing Discipline, 2013.

- Moffat, Osselton, Widdop, Watts, editors. *Clarke's Analysis of Drugs and Poisons Volume I*, 4th Ed. Pharmaceutical Press, 2011. Chapter 23: *Measuring and Reporting Uncertainty* by Mark LeBeau.

9 Quality System Requirements

9.1 Introduction

- The purpose of this section is to provide a uniform Quality Assurance Program for the Toxicology Unit. In combination with the Toxicology Quality Procedures, it is designed to ensure that the parameters of the testing process are routinely monitored in a manner that ensures the reliability of the analytical results. This document is to be used in combination with the crime lab Quality Manual.

- Since most forensic toxicology specimens are limited in quantity or volume, it is important to minimize the need for repeat analysis due to the potential failure of equipment, materials, standards or reagents. The focus of the quality assurance program is to prevent problems before they occur.

- The forensic scientist shall report any unacceptable or anomalous behavior of any analytical system immediately to the Toxicology Supervisor or Quality Manager. It is further expected that appropriate actions will follow as soon as possible and be properly documented. The analyst will initiate an error log workflow in the QMS that will notify the entire unit about an instrument error and the resolution for that error.

9.2 Reagents

- All reagents, chemicals, solutions, etc. used in casework are considered critical items that can affect the quality of testing.

    - Chemicals used in qualitative and quantitative analyses shall be at least ACS reagent grade or better.

    - Solvents shall be high quality, low residue solvents (e.g., HPLC grade, Optima etc.).

    - Water used in reagent preparation should be 18.2 mega Ohm or deionized water from the Millipore (or equivalent) water filtration system.

    - Upon receipt of all reagents, chemicals and supplies that could potentially affect test results, the packing slip will be checked for agreement with items received and this review is documented with the initials/date of the receiver on the packing slip. This packing slip is then scanned and documented for toxicology records in the purchasing workflow inthe QMS. The Toxicology Supervisor should be notified if the received items do not meet quality specifications so action can be taken to correct the problem.

    - The following information shall be recorded for all purchased reagents and reference materials, either on the bottle or in a log with a reference to the bottle:

        - Date of receipt and initials of person receiving the bottle

        - Date opened and initials of person opening the bottle

        - Date of verification (if appropriate) and initials of person performing the verification

        - The expiration date (if applicable)

        - 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.

- All laboratory prepared reagents, solutions and standards shall be prepared using good laboratory practices.

- All prepared reagents forms shall be maintained in a reagent preparation log book. A different final volume of reagent not listed in a particular reagent preparation instruction may be made as long as the final volume prepared is documented in the reagent preparation logbook. Verification of reagents is maintained in the reagent preparation logbook.

- Some reagents must be quality control tested for reliable performance prior to being used on casework (e.g. pH of a buffer verified before use). A 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. This QC can be used as verification that reagents, mobile phases, etc. were accurately prepared.

- All laboratory prepared reagents/solutions will be clearly labeled to include at a minimum the following information: reagent/solutions identity, preparer's initials, date of preparation or lot number, and expiration (if applicable).

- In general, all solutions and reagents (unless otherwise indicated in a specific SOP) may be stored at room temperature for up to one year after the preparation date or when the solution/reagents fail the quality control check or a component in the reagent reaches a manufacturers expiration date (whichever comes first).

- All chemicals and commercial reagents shall be replaced when their stated expiration date or shelf life has expired and/or when they fail the quality check. Expiration dates can be extended if the validity of the chemicals/reagents can be proven through passing quality control checks.

- Chemicals with no stated expiration date will be considered expired 5 years after the received date.

9.3 Blank Blood

- Blank blood is purchased through an external vendor. The toxicology unit is using negative bovine blood from Innovative Research for matrix-matched analysis. The blank blood comes prepared with potassium oxalate and sodium fluoride preservatives. Other sources of blank blood can be utilized as long as equivalent criteria are met.

- The blood containers have a lot number and expiration date. The blood is stored refrigerated, but can be frozen for future use when large volumes are received. The expiration date only refers to cell viability; therefore, the blood can be used past the expiration on the bottle. The expiration for the blank blood is set at one year after the

received date, regardless of the expiration from the manufacturer. Expiration dates can be extended if the validity of the blank blood can be proven through passing quality control checks.

- Each lot of blank blood shall be analyzed for all analytes reported by the unit. The Toxicology Supervisor shall be notified if the lot of blank blood is positive and the bottle shall be clearly identified as having a positive analyte.

- The lot of blank blood shall not be used for assays that tested positive for the analyte of interest.

9.4 Reference Materials

- Whenever possible, certified reference materials shall be acquired from vendors accredited to ISO 17034 standards and includes the supplier's Certificate of Analysis to document traceability, purity, accuracy, precision and homogeneity.

- For instances where traceability cannot be established using certified reference materials, other approved vendors may be used to purchase reference materials.

- Reference materials used in casework are considered critical supplies and shall be purchased from manufacturers/vendors/suppliers approved by the Toxicology Supervisor/Quality Manager.

- Reference materials not verified by the vendor must be verified and documented prior to use or concurrently with casework. Whenever possible, verification should include full mass spectral analysis with comparison to library spectra and the absence of additional/interfering chromatographic peaks.

- Verification data shall be reviewed by the Toxicology Supervisor or designee

- The Certificate of Analysis for purchased reference material is stored in the Toxicology Unit folder within the QMS

- Due to the nature of the work within the Unit, verification of prepared standards from reference material may be performed within a batch of samples when the standard is used as a calibrator or control. A QMS workflow for verification will be initiated and the associated data will be documented within the workflow.

- Reference materials shall be stored in a manner that maintains their quality. In general, powders are stored at room temperature, aqueous solutions are stored at 3-13°C and methanolic standards are stored at -10 to -20°C, unless otherwise indicated by the supplier or in a specific method SOP. All reference materials and reagents should be allowed to come to room temperature prior to use.

- Expiration dates for multi-component reference material solutions prepared in-house should be set for one year from date of preparation or for the earliest expiration of a reference material component. Solutions may be retested to extend the stated expiration date if necessary.

- The expiration dates provided by the manufacturer will be used for materials purchased from an external provider and not altered within the unit. These include alcohol calibrators/QC, UTAK 0.08 controls, , etc. Expiration dates can be extended if the validity of the materials can be proven through passing quality control checks.

9.5 Reference Collections

- Reference collections of data or materials used for identification, comparison or interpretation shall be fully documented, uniquely identified and properly controlled. No changes may be made to purchased reference libraries (e.g. NIST library for GC/MS).

- Data libraries obtained from reputable forensic sources are fully documented and uniquely identified. No changes may be made to these reference collections (e.g. ABSciex Q-trap screening library).

- For in-house libraries, each entry is added and saved in the instrument software. These libraries shall be generated or modified by a qualified scientist or by a designee of the Toxicology Supervisor.

9.6 Pipettes

- Dual diluters fixed volume, and variable volume pipettes shall have their calibration evaluated and certified annually by an approved accredited vendor whose scope of accreditation covers the calibration performed. It is understood that a vendor might not be available at exactly one year from the previous calibration, so the calibration must occur within 3 months of the expiration.

- The pipette calibration certificate shall document the following criteria:
  - The systematic and random error with acceptable limits at each measured volume
  - 5 measurements of each specified volume within the range of calibration
  - The pass/fail status of the pipette

- Repair documentation and calibration certificates generated by the vendor are maintained in the QMS as a workflow. The calibration certificates demonstrate that the pipette has passed QC prior to being placed into or back into service.

- As needed, clean the inside and outside of pipettes with isopropanol, methanol, or a bleach solution.

- If a pipette appears to be out of calibration between normally scheduled performance/calibration checks, the pipette will be repaired/recalibrated by an authorized vendor or taken out of service.

- All pipettes shall be uniquely identified with a serial number and appropriately labeled with the date of calibration and when the next calibration is due.

9.7 Refrigerators/Freezers

- All refrigerators and freezers that are used to store biological evidence or critical reagents are monitored weekly to ensure the appropriate storage temperature. This includes refrigerators and freezers within the section and those used by evidence receiving for the temporary storage of toxicology evidence.

- A temperature log is posted on each refrigerator or freezer.

- A thermometer is placed in each refrigerator/freezer. All thermometers shall be NIST traceable or verified annually against a NIST traceable thermometer.

- Record the thermometer temperature of refrigerators and freezers weekly on the log along with the initials of the analyst performing the check.

- For refrigerators, the temperature should fall between 3-13°C.

- For freezers, the temperature should fall below between -10°C and -20°C.

- 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. Document this adjustment on the temperature log and continue to monitor the temperature of the unit daily for the following week to ensure the thermostat adjustment was effective.

- 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 shall be removed immediately from the affected unit and placed in alternative refrigerators and/or freezers. The refrigerator or freezer will be placed out of service, labeled as such until it can be repaired and the repair shall be documented on the temperature log.

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

- Completed temperature logs for all refrigerators and freezers will be stored as log records in the QMS.

9.8 Heat Blocks/Evaporators

- Heat blocks are generally used for the evaporation of samples.
- With each use, the temperature of the heat block is checked with a thermometer to ensure the temperature falls within the recommended approximate temperature range.
- The thermometer shall be NIST traceable or verified annually against a NIST traceable thermometer.
- Adjust the thermostat as necessary to achieve the desired temperature.
- The temperature should fall within 2°C of the recommended temperature range.
- If the correct temperature cannot be achieved, remove the heat block from service and label it as such until it can be repaired.
- For the evaporators, check the nitrogen flow through the tips. Clean the tips with Methanol or alcohol wipe after use to avoid contamination of samples.
- Documentation of the performance of the heat block/evaporator is evidenced by the acceptable performance of the calibrators and controls with each batch analysis.

9.9 Centrifuges

- All recommended centrifuge speeds are approximate in order to achieve separation; therefore, it is not necessary to conduct a calibration or check to verify speed.
- Clean centrifuge as needed with a bleach solution.
- If the centrifuge needs maintenance or repair, it shall be labeled as out of service until it is repaired and functioning properly.

9.10 Thermometers

- All thermometers used to check temperatures on refrigerators, freezers, heat blocks, evaporators, etc. shall be NIST or NIST traceable, or they may be checked for accuracy against a NIST traceable thermometer annually.
- NIST or NIST traceable thermometers are good for 2 years from their original date of calibration. Purchase a new NIST or NIST traceable thermometer when the unit needs to be recalibrated. Calibration of the NIST traceable thermometer is documented on the temperature log for each refrigerator/freezer.

9.11 Millipore

- The Millipore Milli-Q water filtration system is used to produce both deionized water and high purity Mega Ohm water.

- The Milli-Q system requires monthly, semi-annual, and annual maintenance. Some of this maintenance is conducted in-house and some is conducted by Millipore through a service agreement. It is understood that a vendor might not be available at the exact time the maintenance is required. Therefore, the maintenance must be performed within 3 months of when it is due.

- The specific in-house maintenance is documented on a form located in the Millipore maintenance log book in the toxicology prep room.

- If the Millipore water system is not functioning as expected, it shall be taken out of service and repairs made. During this time, an alternative but equivalent water source must be used. All repairs shall be documented in the log book.

9.12 Fume Hoods

- The fume hoods in the Toxicology Unit will be performance checked annually by an approved outside vendor. The date of performance check and the next due date is located on each fume hood. It is understood that a vendor might not be available at exactly one year from the previous maintenance, so the maintenance must occur within 3 months of the expiration.

- If the fume hood is not functioning properly, it shall be labeled as out of service until repairs can be made.

9.13 Gas Chromatographs

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

- Day-of-Use

  - Perform a quicktune. A hardcopy of the quicktune shall be maintained in the instrument logbook for each month. The maintenance logs will be recorded in a QMS logs workflow once completed. Quicktune results should meet the following criteria:

    - The mass assignments shown in the display should be within ± 0.2 amu of 69, 219 and 502.

    - The peak widths (PW) of the three peaks should be 0.6 ± 0.1 amu.

    - Air and water leaks (masses 28 and 18) should be minimal.

- Nitrogen should be below 15%.
- Electron Multiplier Voltage should be ≤ 2000.
- More specific guidelines can be found in the GCMS quick reference troubleshooting guides located in the instrument log book binders.

- Weekly
  - Run and Etune or Autotune. This must meet the same criteria as the quicktune.
  - Check pump oil level
  - Check Helium tanks
- As needed GC/FID/MS Maintenance:
  - Replace septa/Merlin Microseal
  - Replace Liner / O-ring
  - Replace Gold seal
  - Replace Syringe
  - Clip/Change Column
  - Clean the inlet
  - Check and replace gas cylinders.
  - Ballast Pumps
  - Clean Source
  - Replace Filaments
  - Clean Turbo Pump
  - Replace Pump Oil
  - Replenish PFTBA
  - Clean HED

- Clean FID

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

- Retain instrument verification documentation in the instrument logbook or electronically.

- Hydrogen generator

  - Add deionized water weekly or as needed.

  - Change deionizer bags at least annually.

9.14 New Instrument Installation

- Complete the "Equipment List" QMSworkflow to document the location in the laboratory, type of instrument and all serial numbers associated with the components of this instrument.

- Obtain documentation from the instrument service representative demonstrating that the instrument performs to the manufacturer's specification. This is stored in the QMS.

- Load methods, reports and libraries and test their functionality.

- After methods have been loaded or created, run negative controls, 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).

- Retain instrument verification documentation inthe QMS.

- The verification shall be sent to the Toxicology Supervisor for approval prior to placing the new instrument into service.

- If the instrument does not meet expectations or acceptance criteria, label it as "not in service" and notify the toxicology staff. The vendor should be contacted (if necessary) to make arrangements for instrument replacement or maintenance.

9.15 Glassware

- Volumetric glassware used to prepare calibrators/standards shall be NIST traceable and visually inspected prior to use.

- Volumetric glassware shall be replaced every 10 years.

- Glassware can be washed with an approved detergent and rinsed with high purity or deionized water. Additionally, the dishwasher may be used to clean glassware.

9.16 Positive-Pressure Manifolds (PPM)

- The PPM should be operated at 85psi.
- Check the system for leaks. Hissing or squeals will indicate a ruptured gasket.
- Clean the surface of the manifold as needed to prevent corrosion and contamination.
- Replace the gasket as needed.
- Lubricate the manifold arm as needed.
- The PPM should be placed "out of service" if not functioning properly and maintenance performed.

9.17 Safety

- Appropriate PPE shall be worn while preparing samples for analysis. This includes a lab coat, gloves, and safety glasses. All chemicals shall be handled with caution. The lab area should be cleaned with a bleach solution after preparation is complete.

9.18 References

- Millipore Milli-Q Integral Water Purification Systems User Guide.
- Positive Pressure Manifold Operation and Maintenance Form.
- Agilent's Troubleshooting and Operations Manuals.

Appendix A - Commonly Used Abbreviations

The following is a list of abbreviations commonly used in the toxicology unit. This list has been generated to assist the forensic scientists/technicians in the interpretation of case file notes, labels, data files, etc. This is a comprehensive list; however, the list does not encompass all abbreviations that can be used within the unit. Each analyst has the flexibility to use these and other abbreviations, but they should be easily interpretable within the context of the subject material. It is encouraged that analysts in the toxicology unit refer to the abbreviations list to promote consistency of documentation within the unit.

General Abbreviations

Abbreviations	Definitions
A/N	Acidic/Neutral Drugs
ABC	Alcoholic Beverage Commission
Abs	Absorbance
Acids	Acidic Drugs
ACN	Acetonitrile
Admin or ADM	Administrative
AI	Amphetamines/Illicit Mix
AIM	Acetone/Isopropanol/Methanol control
Alc	Alcohol
Approx, ~	Approximately
Ave, Avg	Average
Bases	Basic Drugs
Blk	Blank
BAC	Blood Alcohol Concentration
Bld	Blood
BO	Benzodiazepine/Opiate Mix
Btl	Bottle
Cal	Calibrator
c/o	Carryover
Cer	Cerilliant
Cert	Certified
CL	Crime Lab
Cntl, Ctrl	Control
Conc	Concentration
Conf	Confirmed
Cmpds	Compounds
CRM	Certified Reference Material
% CV	Coefficient of Variation
D (followed by a #)	Deuterated compound
DCM	Dichloromethane
DI	Deionized Water
Dil	Dilution/diluted
ELISA	Enzyme Linked Immunosorbant Assay
EA	Ethyl Acetate
EPI	Enhanced Product Ion
eV	Electron volts
Evid	Evidence
Ext	Extraction/extracted
Exp	Expiration
FID	Flame Ionization Detector

FS	Forensic Scientist
FT	Forensic Technician
g	gram
G%, Gm%	Gram %, Gram per 100 Milliliters
GC	Gas Chromatograph/Gas Chromatography
GC/MS	Gas Chromatograph/Mass Spectrometer
H2O	Water
HCl	Hydrochloric Acid
Hex	Hexane
HPLC	High Pressure Liquid Chromatography
HS	Headspace
ID	Identification
Imp	Impurities
Info	Information
Int, integ	Integration
IPA, 2-Prop	Isopropyl Alcohol
IS, ISTD, Int Std	Internal Standard
LC/MS	Liquid Chromatography/ Mass Spectrometry
LC/MS/MS	Liquid Chromatography/ Tandem Mass Spectrometry
LIMS	Laboratory Information Management System
Liq	Liquid
LL, LLE	Liquid/Liquid Extraction
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LOQ	Limit of Quantitation
MeOH	Methanol
mg	Milligram
MNPD	Metro Nashville Police Department
MPA	Mobile Phase A
MPB	Mobile Phase B
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
MSD	Mass Spectrometer Detector
Met	Metabolite
NAOH	Sodium Hydroxide
NH4OH	Ammonium Hydroxide
Neg	Negative
ND	None Detected
NDD	No Drugs Detected
ng/mL	Nanograms per Milliliter
NIST	National Institute of Standards and Technology
NR	Needle Rinse
Orig	Original
PFTBA	Perfluorotributylamine
Pk	Peak

PR	Present
Prep	Prepared
PM	Preventative Maintenance
PPM	Positive Pressure Manifold
Pos	Positive
Quant	Quantity/Quantitation
Qual	Qualitative
QA	Quality Assurance
QC	Quality Control
QNS	Quantity Not Sufficient for analysis
QQQ	Triple Quadrupole
Qs	Add quantity sufficient to bring up to volume
Q1	Quadrupole 1 (LC/MS/MS instrument)
Q3	Quadrupole 3 (LC/MS/MS instrument)
Recv, Recd	Received
RF	Ratio Failure
RSD	Relative Standard Deviation
RT	Retention Time
RRT	Relative Retention Time
Scrn	Screen
SD	Standard Deviation
SIM	Selected Ion Monitoring
Solv	Solvent
SPE	Solid Phase Extraction
SS	Secondary Standard
TIC	Total Ion Chromatogram
Tox	Toxicology
TTM	Technical Training Manual
TPM	Technical Procedures Manual
ug/mL	Micrograms per Milliliter
ULOL	Upper Limit of Linearity
ULOQ	Upper Limit of Quantitation
U of M	Uncertainty of Measurement
UV	Ultraviolet spectrophotometer
UV-vis	Ultraviolet/visible spectrophotometer
Vol	Volatile
V/V	Volume per Volume
w/	With
w/o	Without
WB	Whole Blood
WT	Weight
W/V	Weight per Volume
Yr	Year
(+)	Positive
(-)	Negative

↓	Below
↑	Above

Drug Names

Abbreviations	Definitions
6AM, 6MAM	6-monoacetylmorphine
Ace	Acetone
Ace Fent	Acetyl Fentanyl
Acet	Acetaminophen
Alp, Alpraz, Alpz	Alprazolam
Amit, amitrip	Amitriptyline
Amo, amobarb	Amobarbital
Amp	Amphetamine
Amps	Amphetamine drug group
Barb, barbs	Barbiturates
BZE, BE	Benzoylecgonine
Bromphen	Brompheniramine
Benzo,benzos, BZO	Benzodiazepines
BUP	Buprenorphine
Butal	Butalbital
Cannabs	Cannabinoids
Carboxy-THC, THCA, THC-COOH	THC carboxylic acid
Cariso	Carisoprodol
Cbd	Cannabidiol
CE	Cocaethylene
Chlordiaz	Chlordiazepoxide
Chlorphen	Chlorpheniramine
Cit	Citalopram
Clonaz	Clonazepam
COC	Cocaine
Cod	Codeine
Cyclo, Cyclobenz	Cyclobenzaprine
Dextro	Dextromethorphan
Diaz, DZ	Diazepam
Dihydro	Dihydrocodeine
DFE	Difluoroethane
Diphen	Diphenhydramine
Dox	Doxepin

Doxyl	Doxylamine
Ephed	Ephedrine
Estaz	Estazolam
ETOH	Ethanol
Fent	Fentanyl
Flunit	Flunitrazepam
Fluox, Flx	Fluoxetine
Fluraz	Flurazepam
Gaba	Gabapentin
GHB	Gamma hydroxybutyrate
Hydrocod	Hydrocodone
Hydromorph	Hydromorphone
Ill	Illicits
IPA	Isopropyl alcohol or Isopropanol
Loraz	Lorazepam
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MDN	Methadone
MDPV	3,4-methylenedioxypyrovalerone
MeOH	Methanol
Meper	Meperidine
Mepro	Meprobamate
Meth, Methamp	Methamphetamine
Midaz	Midazolam
Morph	Morphine
Mthphen	Methylphenidate
Nordiaz, NDZ	Nordiazepam
Norfent	Norfentanyl
Norfluox, Nflx	Norfluoxetine
Normep	Normeperidine
Norpropoxy, Nppx	Norpropoxyphene
Norhydro	Norhydrocodone
Noroxy	Noroxycodone
Nortrip	Nortriptyline
n-Prop	n-Propanol
Opi	Opiate(s)
Oxaz	Oxazepam
Oxy, Oxycod	Oxycodone
Oxymorph	Oxymorphone
Parox	Paroxetine
PCP	Phencyclidine
Pentobarb	Pentobarbital
Pheno, Phenobarb	Phenobarbital

Pregab	Pregabalin
Phent	Phentermine
Prometh	Promethazine
Propoxy, Ppx	Propoxyphene
Pseudo	Pseudoephedrine
Seco, Secobarb	Secobarbital
Sert	Sertraline
Sufent	Sufentanil
Tapent	Tapentadol
TCA	Tricyclic Antidepressants
Temaz	Temazepam
THC	Tetrahydrocannabinol, delta-9-THC
THC-OH	11-Hydroxy-delta9-THC
THC-COOH	11-nor-9-Carboxy-delta9-THC
Triaz	Triazolam
Tram	Tramadol
Traz, Trazdn	Trazodone
Venla	Venlafaxine
Zolpid	Zolpidem
Zopic	Zopiclone
Zalep	Zaleplon