1.0 Principle:

Samples received for analysis by the Toxicology Unit are maintained as case evidence by the laboratory with complete documentation of Chain of Custody (COC) in the DSS LIMS system. All cases are assigned unique alpha-numeric identifications in JusticeTrax. Cases are entered into JusticeTrax by the Evidence Receiving Unit, and placed in storage, in a secure evidence handling area; until transferred to a member of the Toxicology Unit. The toxicology analyst will transport the samples to the Toxicology laboratory and store them in the appropriate evidence refrigerator (documenting the COC in JusticeTrax).

All cases are itemized in Justice Trax based on the number and types of samples submitted. In general the unit uses the preset itemization that is the default in JusticeTrax. Barcode labels are printed based on the itemization for the samples. The analyst that itemizes the case verifies that the case is sealed appropriately and that the case demographics on the paperwork match that on the evidence. The analyst then uses the barcodes to label the itemized samples. The samples are then sealed and transferred back to locked storage until needed for analysis.

If at any time, the sample suitability is in question, the sample description does not correctly reflect the evidence, or more detail as to the type of analysis needed is required, the laboratory shall consult the submitting agency and keep a record in the case file.

Analysts assigned to a case remove the needed case(s) from the storage location and document the transfer in JusticeTrax. While under analysis the evidence boxes/bags need not be sealed, however they will remain securely stored within the unit. Following completion of the analytical process (see section 6.5 below for definition), the evidence is sealed and is stored in a locked refrigerator, the analyst prepares a report, and the case receives a technical review by the unit lead or designee. The final report in the case is signed by the analyst and reviewer (or printed with electronic signatures); the case folder is then filed.

2.0 Safety Precautions:

Cases in the Toxicology section will generally be biological fluids (urine or blood), with occasional poisoning cases which can take most any form. Universal precautions must be taken in this section when handling case materials. Other hazards in this section are chemicals used with the specific methods; see the individual SOPs for this safety information.

3.0 General Toxicology Procedures:

3.1 Case Receipt; any analyst in the Toxicology section may retrieve new cases through Evidence Receiving (or other sections in the case of shared evidence). The Evidence Control Officer (ECO) or appropriate individual will transfer cases to the analyst through Justice Trax. The analyst transports the evidence to the Toxicology Unit. The analyst accesses JusticeTrax in the laboratory and transfers the case materials to the proper storage refrigerator. Each time a case is accessed by an analyst it must be tracked in Justice Trax. PINs will be used by each analyst involved in an evidence transfer.

3.2 Discrepancies; if an analyst notes a discrepancy in a case number, source name or other information this should be documented in the case file and brought to the attention of the Unit Lead and/or to the Evidence Control Unit Lead. Depending on the nature of the discrepancy the submitting agency may need to be contacted to clarify the issue. All information concerning discrepancies will be noted in the case file (and if appropriate in the synopsis section of the case in LIMS).

3.3 Case Labels; when the case is itemized in JuxticeTrax barcode labels are generated. These are placed on the appropriate samples by the individual itemizing the case. In general the unit sub-itemizes samples using the default set in JusticeTrax. When samples are sub-itemized in another unit the designation assigned by that unit will be used.

All case aliquots or extractions of case materials are properly identified by marking the test tube or specific container with the case number and the specific item number designation. For materials prepared for instrument analysis it is acceptable to label the sample tube with another designator (such as auto sampler position number).

3.4 The Laboratory shall have appropriate facilities to safely and securely store evidence and protect it from loss or damage during analysis. These facilities (i.e. refrigerators and lockups) shall be monitored for proper function to protect the condition of the evidence. See SOP Tox 14 section E.

4.0 Case Analysis; the types of analysis in the Toxicology Unit are alcohol (or volatile), drug and other poisons. Various laboratory instruments are used in the analysis, which can include but are not limited to EMIT, HS/GC, GC, GC/MS or LC/MS. These techniques may be used for screening or confirmatory analysis. Any sample going through the section may have all types of testing or just specific testing depending on the nature of the case and why it was sent to the laboratory.

Throughout the process of samples being accessed the chain of custody will be maintained in Justice Trax. Analysts will use their secure PIN to move cases from storage sites into their custody and back again. Since the majority of cases in the Toxicology Unit are not assigned to a single analyst it is not anticipated that an analyst will have a sample in their custody overnight.

4.1 Case types in the Toxicology unit are, in general, driving under Influence (DUI), Drug Facilitated Sexual Assault (DFSA), Motor Vehicle accidents, and Poisonings. Each case type flows through the section in its own specific manner based on the needs of the sample.

4.1.1 **DUI** cases are analyzed by EMIT for alcohol and general drug screening. Positive alcohol samples are then run by HS-GC for quantitative alcohol results. DUI samples then may have drug confirmation tests based on the EMIT and Alcohol results, or testing may stop and a report generated.

DUI samples (including non-fatal motor vehicle accidents) will be evaluated after the EMIT is run. A report may be generated based on the volatile and EMIT results only; indicating that confirmatory testing is available upon request. When this is done the report will clearly state that the results are from an EMIT screening method and that if confirmation is required the customer must contact the laboratory within eight weeks to order the needed testing.

> Example (similar wording is acceptable): Presumptive screen testing was positive for Cocaine Metabolites, Phencyclidine and Cannabinoids.
>
> Please contact the laboratory at 203-427-4098 within 8 weeks if confirmatory analysis is required.

If a request for confirmatory analysis is made the person receiving the request will annotate in LIMS who made the request, the date and contact information (form TX-19:1 will be completed). This form will be forwarded to the unit lead (or their designee). A request will be added in LIMS and added to the appropriate work list(s).

General Guidance:

For DUI cases a report may be issued after the Volatile analysis and EMIT screen, this is dependent on the results and case scenario.

The Deputy Director or Unit Lead (or designee) will evaluate the case after the Volatile and EMIT analysis and may require further work based on the specific case scenario. When further work is required the case file will be documented to include the reasoning.

Some case scenarios that may require further analysis (see TX-20 EMIT for further guidance) include:

- A sample with a 0.00ug/dL alcohol level and an EMIT negative: WAN/BDS analysis will be performed.

- A sample where the arresting officer specifically asks for a substance that is not part of the normal EMIT panel (i.e. a synthetic cannabinoid, or bath salt).

The individual assessing the case will note the reason, in the case file, for continuing work.

4.1.2 **Drug Facilitated Sexual Assaults** in general these cases will initially be analyzed by EMIT for Alcohol and drug content. Positive alcohol samples are quantitated by HS-GC. These cases may be screened for GHB and or sedative hypnotics like benzodiazepines or zolpidem depending upon case history and time of assault and specimen collection. All DFSA samples have a general unknown drug screen analysis performed regardless of alcohol and/or EMIT results.

4.1.3 **Motor Vehicle Accidents:** In general, these cases involve a fatal or potentially fatal accident. The laboratory receives specimens from the driver(s) involved, but not killed. These samples are analyzed for alcohol and drugs by EMIT. Positive alcohol samples are quantitated by HS-GC. Regardless of the alcohol results or drug screen results a general unknown drug screen analysis will be performed. Further analysis may be indicated based on the results of the EMIT and general unknown drug screen.

4.1.4 **Poisoning** cases will be assessed by the Deputy Director with the unit lead and/or the analysts involved. Case history, nature of the submitted evidence, specific request from the submitting agency will be used to guide the analyses. There is no general case flow for these samples since they could range from suspected drug poisoning to heavy metals or any other variety of possible poisoning methods. The Deputy Director may suggest sending a poisoning case out if it is not in the scope of the units testing abilities, such as heavy metal testing. In these cases the submitting agency will be contacted to gain their approval.

4.1.5 **Proficiency Testing** cases will be analyzed similar to actual cases in order to check the Laboratory system.

4.2 **Alcohol Analysis**: in general and for the majority of cases alcohol analysis will be performed as part of the EMIT screen. Samples identified as positive for alcohol by EMIT are

batched together for quantitative analysis by HS-GC. Analysts retrieve samples from their storage location, track this in Justice Trax and perform the analysis per the specific SOP. After completing a batch, the analyst gives the batch to the Unit Lead or their designee for a batch technical review. Once a batch is reviewed and accepted, the results are entered into JusticeTrax and a report is generated. In general, alcohol reports are generated separately from drug reports, so it is common for any given case to have two final reports associated with it; one for the alcohol results and one for the drug results. When reports are issued based on volatile results and EMIT, a single combined report may be issued.

4.3 **EMIT**; this is a general screen for alcohol and drugs performed on all DUI samples and on some other sample types, such as sexual assaults and fatals. When the EMIT analyst completes their analysis and prepares the associated batch paperwork, they give the batch to the Unit Lead or their designee for a batch technical review. After the batch is reviewed the paperwork is returned to the analyst. The analyst then files the paperwork into the appropriate case files and files the batch in the Toxicology laboratory. The results are then entered into JusticeTrax. Based on the case finding and type of case, the milestones may be updated to draft complete so a draft report may be generated, or the file is returned to the unit for further analysis. When confirmatory drug work is required the analyst adds a request in JusticeTrax. The analyst will follow the guidance of SOP TX-20 EMIT to determine if a case requires additional work. If there are additional case circumstances they may also consult the Unit Lead or their designee to determine if additional testing is appropriate.

4.4 **GC/MS or LC/MS confirmatory analysis**; confirmatory analysis are performed in batches following the SOP for the specific analysis needed for the specific case. The analyst determines the needed tests based on the EMIT results or the specific case type and information. When a batch is completed the analyst gives it to the Unit Lead or their designee for a batch technical review. When the batch technical review is complete and the work accepted the analyst then enters the findings into JusticeTrax; this is done until all needed work is completed for the case. The last analyst entering in a result on a case will draft complete the case, print the draft report and give the case file to the Unit Lead or their designee for the Final Technical Review. Analysts performing confirmatory tests need to review individual cases for completeness to know at what point no further work is required and the report can be generated.

> Note: Case jackets in this section will have initials from multiple analysts; each batch will have the original analyst's initials (these may be photocopied initials). It is not expected that one analyst take ownership of a case and re-review each batch that has made up the case.

5.0 Reagent Validation;

5.1 When a new lot of a solvent or reagent is needed, the new solution will be used to extract case materials as stated in the SOP of the specific procedure. A blank and any controls, if applicable, will also be run. The analyst will verify that the solution worked for the stated method and that there were no interfering or unexpected agents found. (Note that some solvents contain trace levels of substances such as formaldehyde in methanol which is inherent to the product; if these do not affect the test they are acceptable). Documentation for the acceptability will live with the batch documentation.

5.2 A solution is considered validated and acceptable for use when;

5.2.1 The solution is shown to have worked in the procedure

5.2.2 The solution blank is shown not to contain contaminants that will interfere with the procedure.

5.2.3 Documentation is complete, including:

5.2.3.1 Annotating the validation of the reagent on the batch run sheet and having the batch technically reviewed.

5.2.3.2 Complete the 'Reagent Log' book, this states the name of the reagent/solution, lot number, the date made by who and date validated. The lot number may be the date made and prepares initials for reagents made in-house.

5.2.4 The solution is marked with a green sticker, with which method the solution is validated for, the date validated (the batch date) and the analyst's initials.

Solvents and some other substances are purchased in multi-bottle lots; once one bottle from the lot is found to be acceptable for a procedure then all the bottles in the lot can be marked as validated.

Bottles with working solutions will be labeled with:

Solution name
Lot number (which is the date made for reagents made in-house)
Date filled or made (if only a transfer from a purchased solution or a larger batch of a made reagent)
Expiration date (if different then the standard one year from date of validation)
Analyst initials
Green label (with the validation information).
Required safety information

6.0 Case Reporting;

6.1 Case reports are generated through the LIMS (JusticeTrax) program.

6.2 Once a report is generated and reviewed by an analyst; a technical and administrative review are performed prior to the signed case report being released.

6.2.1 Technical review; this is performed by the Deputy Director, Unit Lead or their designee. The technical reviewer is responsible to review the case to determine if the conclusions made by the analyst(s) are supported by the analytical data in the case file. They are required to make sure that all data entered on the report is appropriate and that no findings are left out. They are not required to re-review batches of work; they may if they feel it is needed to understand the final report. The technical reviewer may request additional work based on the case scenario and findings.

6.2.2 Administrative review; this consists of a review of the case folder to determine that all pages are initialed by the analyst and that the case number is on all pages. The report is reviewed to check that the inputted clerical data is correct including the agency name, source name, and agency case number.

The technical and administrative reviewer uses form TX-5.1 to document the technical review. Refer to SOP TX-5 'Case Documentation' for further guidance in performing a technical or administrative case review.

6.2.3 The Case report is signed and dated by the analyst(s) and technical reviewer. The report is copied and distributed as designated in SOP TX-5 (Case Documentation). The case file is filed within the Toxicology Unit.

6.3 Uncertainty:

Procedural Uncertainty is reported with all quantitative results, and is evaluated annually for each analytical method. The overall uncertainty for the procedure ("expanded uncertainty") is calculated based on an evaluation of control performance (variance) and bias, documentation of uncertainty in materials used to prepare standards used in calibration, and the uncertainty introduced in the process of standard preparation. Uncertainty associated with the performance of the procedure, and the preparation of controls is captured in the process of evaluation of the control variance. Note: Since there may be limited "historical" data generated each year it may not be appropriate to recalculate the uncertainty for some methods. In these cases the annual evaluation will clearly state that enough new data has not been generated to recalculate the uncertainty.

Documentation of the calculation (and annual evaluation) of uncertainty for each procedure is maintained in the "Procedural Uncertainty" Notebook, maintained in the laboratory. Included in this notebook is documentation (or appropriate references) of reference materials and equipment utilized in the procedure that could affect the quantitative accuracy of the method.

Notes:

1. Uncertainty is reported in the same units as the analyte concentration (e.g. ug/g, g/dL), +/- the expanded uncertainty for the procedure, using a coverage factor (k) = 2 (corresponding to a 2SD range, and 95.45% confidence interval). (Note: Per State Statute (CT 14-227a) Ethyl Alcohol is reportable as a "weight percent", (grams/100 mL). Uncertainty for this procedure will be expressed in the same units reported, grams ethanol/100 mL blood, expressed as a percent.

2. Equipment:
    a. Equipment Potentially Impacting Procedural Uncertainty
        1. Analytical Balance (Volatiles, GHB)
        2. Automatic Pipettor/Dilutor (Volatiles, GHB)
        3. Automatic Pipettes (Toxicology Procedures)
        4. Mass Spectrometers (Toxicology Procedures)

    b. Calibration of the Headspace-GC is not a factor in quantitative performance.

    c. Calibration of Mass-Spectrometer instrumentation (GC- or LC-) can be a factor in quantitative performance. Instrumental mass-axis calibration is adjusted as necessary during tuning procedures performed on each day of use. Mass-axis calibration is essential for normal batch processing (mass-axis calibration error would result in calibrator, and/or internal standard spectral match failure, leading to batch rejection and identification of problem.) Tuning documentation is maintained in an instrument-specific notebook.

    d. Pipettes are evaluated on an annual basis, by an ISO-certified (17025) vendor. Documentation of Pipette performance is maintained for each individual pipette in the "Validation of Miscellaneous Equipment" notebook, in the Toxicology Unit as per SOP TX-14; "Maintenance of General Equipment."

e. Balance calibration is performed annually and documented in the balance logbook, kept by the balance in the Toxicology Unit. Balance calibration must be performed by an ISO-accredited (17025) facility.

f. Reference masses are evaluated at minimum every five years by an ISO certified (17025) vendor and are verified annually in-house. Documentation of mass evaluation is maintained by the Quality Section. (See GL-21 General Laboratory Equipment' for further guidance).

3. Reference Materials: Reference materials must be CRM provided by an ISO 17025 or equivalent accredited vendor. Materials unavailable from ISO accredited providers must be evaluated and validated for use by the laboratory as per SOP TX-11; "Standard Validation". Documentation of standard materials, including (as applicable) the COA and any additional validation information is maintained in the "Standard Validation Logbook" kept in the Toxicology Unit.

4. Providers of CRMs or Reference materials will be assessed to assure that they are a NMI or reference material producer that is ISO34:2009 accredited through ILAC – with appropriate scope of accreditation.

5. Accredited vendors used for calibration services will be assessed to assure that the service required is covered under the vendor's scope of accreditation.

6. For analytes where there is no historical data to determine uncertainty, (and the analyte does not fall into a class of drugs where uncertainty has been established) uncertainty will be reported based on the response of the batch controls or as documented in the 'Procedural Uncertainty' logbook.

6.4 Toxicology abbreviations and symbols.

DUI	Driving under the influence
DOC	Department of corrections
DFSA	Drug facilitated sexual assault
EtOH	Ethanol
MeOH	Methanol

IPA	Isopropanol
RT	Retention time
EMIT	Enzyme Multiplied Immunoassay Technique
GC MS	Gas Chromatography Mass Spectroscopy
HS GC	Headspace Gas Chromatography
IR	Infrared
UV	Ultravisible
LC MS	Liquid Chromatography Mass Spectroscopy
TX	Toxicology
CS	Controlled Substances
WAN	Weak Acid Neutral
BDS	Basic Drug Screen
BL	Blood
UR	Urine
CHEP	Cyproheptadine
DI H2O	De ionized Water
Neg	Negative
NDD	No Drugs Detected
SA	Sexual Assault
COC	Chain of Custody
Coc	Cocaine
BE	Benzoylecgonine
Op	Opioids
THC	Delta-9-Tetrahydrocannabinol
Benzo	Benzodiazepine
SMA	Sympathomimetic Amines
GHB	Gamma Hydroxybutyrate
COA	Certificate of Analysis
CRM	Certified Reference Material

6.5 Definitions:

6.5.1 **Sample, Evidence and Case** are all terms used to identify the biological fluid or other evidence submitted to the laboratory for testing in the section.

6.5.2 **Aliquot**, a portion of sample isolated from the whole for analysis in any of the section procedures.

6.5.2.1 The person taking the aliquot is listed in the LIMS chain of custody and performs testing on that portion. It is acceptable for an analyst to take multiple aliquots from a sample for testing without sub-itemizing in LIMS if the analyst maintains custody of the

aliquots and if they are the analyst that uses the aliquot for testing. When multiple aliquots are taken at one time for testing at a later date the analyst will note this in the case documentation.

6.5.2.2 If an analyst takes multiple aliquots to distribute to other analysts for testing, the aliquots must be sub-itemized in LIMS and the chain of custody maintained in the same manner as the parent item.

6.5.3 **Work product**, portions of samples that are in the process of analysis (i.e. an aliquot that is in the process of solid phase extraction). The COC does not need to be maintained for work product.

6.5.4 "**Completion of Analytical Process**" (or analysis is complete),

6.5.4.1 For cases requiring confirmatory work, "completion of analytical process" is defined as after all confirmatory testing is complete. (Note: this process is not to exceed 6 months).

6.5.4.2 For cases reported after the EMIT Screen the completion of analytical process is at this reporting step. Noting that the analytical process may resume if further testing is requested by the submitting agency.