

1. Scope

This procedure provides general guidelines for the receipt, analysis, and disposition of toxicological evidence for cases assigned to the Toxicology Unit (TU) within the Chemical Analysis Section (CAS) of the Division of Scientific Services' (DSS) forensic laboratory. These guidelines are based on recommendations set forth within a number of reference documents such as, but not limited to:

- DSS laboratory Quality Manual procedures
- ANSI National Accreditation Board (ANAB) AR3125 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements
- Society of Forensic Toxicologists (SOFT)/American Academy of Forensic Sciences (AAFS) Forensic Toxicology Laboratory Guidelines
- American Board of Forensic Toxicology (ABFT) Laboratory Accreditation Manual
- Drug Abuse Handbook
- Poison Detection in Human Organs
- Introduction to Forensic Toxicology
- Principles of Forensic Toxicology, Handbook of Analytical Toxicology

2. Equipment/Materials/Reagents

Not applicable. Guidance for preparing reagents and the utilization of instruments/equipment may be found in specific toxicological procedures.

3. Standards and Controls

Not applicable. Guidance for preparing standards and controls (e.g., positive and negative) may be found within specific toxicological procedures.

4. Calibration

Not applicable. Guidance for calibration may be found within specific instrumental procedures.

5. Sampling

The most common types of toxicological specimens for antemortem analyses are urine and blood (sometimes plasma or serum will be submitted). For postmortem analyses blood, urine, and/or vitreous humor are often analyzed (if plasma/serum are submitted they may be treated like blood specimens and will be analyzed accordingly). Prior to sampling

containers should be inverted or swirled to ensure homogeneity. If blood samples are found to be clotted, such clots may need to be removed or homogenized/broken-up (as best as possible) before sampling occurs. The determination of whether blood clots will be removed or analyzed and the process for ensuring homogeneity of clotted samples will be done on an as-needed basis and will involve a Lead Examiner or higher. Appropriate case notes will be taken for documentation of events.

Blood samples may be received in multiple tubes. When blood has been reportedly collected at the same time interval and from the same location/person, and has been in the same type of test tube (i.e., grey top), it may be considered the same item and be given the same item number during accessioning (e.g., Item #: 001-001 – blood within two (2) red top tubes). In these circumstances the tubes can be further labeled to distinguish the tubes from one another. Any sampling from the separate tubes will be described within applicable case note documents by the analyst(s) who handles the evidence. Situations where it is not clear whether multiple tubes of blood should be considered one item or multiple items will involve the Lead Examiner or higher for a decision to be made. Any clarification or justification for such actions and/or decisions will be appropriately recorded.

6. Procedure

6.1. Sample Collection, Receipt, and Transfer

The proper selection, collection, and submission of biological specimens for toxicological analyses is important for the scientifically sound interpretation of analytical data. There are recommended minimal volume amounts for specific types of specimens in order to adequately perform routine toxicological examinations (e.g., human performance cases: blood = 15 mL ; urine = 100 mL). However, specimens may be limited and submitting agencies may not be able to obtain recommended minimal amounts. In cases where limited sample amounts are received by the DSS laboratory, the type and amount of specimen may influence which analyses will be performed. In such situations analysts will work with Lead Examiners, and possibly the submitting agencies, to decide which analytical path will be pursued.

When blood is analyzed for containing certain drug classes (e.g., cocaine, ethanol) it is advantageous if the specimen is mixed with chemicals so as to enhance the stability of analytes. This can be accomplished by using specimen tubes that contain preservatives and/or anticoagulants. For most toxicology cases, the preferred collection tube is a grey-top Vacutainer® which contains a mixture of sodium fluoride and potassium oxalate. Expiration dates on such tubes are only for vacuum integrity and do not reflect on the quality of the tube or its components after samples have been captured within the container.

Containers with specimens collected from living persons should be found labeled with appropriate information (e.g., source name, time/date of collection). Biological specimens should be submitted to the DSS laboratory in separate individual packages, under proper [evidentiary] seal, with appropriate warning labels, under a single communication (i.e., Request for Analysis (RFA) form), and should be submitted in a refrigerated or frozen condition. Typically such evidence is found submitted within some sort of kit and/or paperwork (e.g., DUI kit, CT400 kit, DPS-0009-C, copy of medical records).

Such evidence will typically be received through the Evidence Receiving Unit of the DSS laboratory. Proper seal of evidence refers to a condition of packaging which ensures evidence is prevented from cross contamination, there is no sample loss, and any attempt at deleterious change of the evidence would be noticeable.

After initially receiving a toxicology case the evidence will be inventoried and information will be entered into LIMS-Plus (JusticeTrax (JT)). The information can include, but not be limited to: type of specimen(s) received, any labeling present on the containers, the approximate specimen amounts, and any damage to or leaks from the containers. The specimen containers will be labeled with appropriate information from LIMS-Plus (e.g., item number(s) and the Laboratory/Case Number). Any problems or issues with evidence will be addressed at the time of inventory, will be documented appropriately, and can involve a Lead Examiner, Assistant Director, Deputy Director, or designee. Pictures may be taken to document the evidence and any picture should contain the lab number, the date, and the analyst's initials. Pictures will be included within appropriate case files (LIMS-Plus or hardcopy). Contributors may be contacted, if appropriate, for minor issues or discrepancies within cases, and will be contacted for major issues within cases (e.g., sample loss or sample integrity issues).

Evidence transfers will involve adequate chain-of-custody (CoC) documentation and will be documented within the laboratory information management system (LIMS) software (e.g., LIMS-Plus). If evidence is transferred to multiple analysts for the purpose of aliquoting samples then evidence does not necessarily need to be under proper seal between such transfers (evidence must be free of sample loss and free of possible contamination). Any transfer of evidence possession needs to be tracked with respect to CoC documentation to ensure that proper accountability is recorded among analysts. If aliquoted portions of a sample are to be consumed during analysis then each portion can either be considered the same item from which it originally came (and the same item number can be used) or it can be sub-itemized (usually describing why it was used). If a different analyst will be taking control of an aliquoted portion then sub-itemization will be performed.

For example: suppose Item #001-001 blood is to be tested for volatiles by Analyst A and will then be tested for drugs by Analyst B. In this scenario Analyst A takes possession of Item #001-001 and aliquots 0.5 mL of blood into a headspace vial for

their own work. To assist their co-worker Analyst A then aliquots 0.5 mL of Item #001-001 blood into a test tube for future drug screening by Analyst B. The aliquoted [0.5 mL] drug screen sample will be sub-itemized by Analyst A as Item #001-001-01 (description may be: 0.5 ml BZ/OP Quant aliquot). Analyst B comes at a later time and physically takes Item #001-001-01, appropriately updating the information within LIMS-Plus as a transfer Item #001-001-01 from the refrigerator to themselves. If there was no more of Item#001-001-01 and it was to be consumed during the drug analysis portion, then the aliquoted Item #001-001-01 would be updated within LIMS-Plus as being consumed in analysis.

In the unusual circumstance that an extract from a specimen will be retained (e.g., all the blood has been consumed and the extract needs to be saved), then the extract will be sub-itemized (e.g., Item #: 001-001-01-01) and recorded within the CoC of LIMS-Plus. Extracts from situations wherein biological evidence has been exhausted should be retained until directed otherwise.

6.2. Sample Storage

Due to the nature of biological material, specimens will be kept refrigerated or frozen and under proper seal when not under active examination. Refrigerators and freezers are located in the Toxicology Unit for evidence storage and shall be appropriately secured within a lockable system. Other areas, if acceptable for evidence storage, may be used to store evidence (e.g., refrigerators and freezers in other areas of the laboratory) and will have similar lockable systems. Use of tamper-evident devices will be employed (e.g., evidence tape, security tags).

6.3. Analytical Schemes for Toxicology Testing

Forensic toxicological examinations are conducted on specimens in order to detect a wide range of drugs and/or other substances. The Lead Examiner, Assistant Director, Deputy Director, or designee will be responsible for evaluating cases prior to reports being issued in order to ensure that all requests have been completed/addressed, good case management has occurred, and quality has been maintained. Refer to specific case types for more guidance. If evidence with limited volume or multiple types of evidence are received (e.g., multiple blood tube types, multiple serum tubes), a lead Examiner (or higher), possibly involving the submitting agency's representative, should be consulted so as to create an analytical scheme directing what evidence will be analyzed so as to maximize efficiency and completeness.

Pertinent case histories should be reviewed during both the accessioning process and when cases are being completed (i.e., technical reviews). For most routine DUI-related cases analysts should only need a brief understanding of the history within requests. Certain cases (e.g., sexual assaults) may require a more thorough review of case history so that adequate analytical plans are developed and followed.

Professional judgment will be used to determine the sequence of tests which will be

performed. The general analytical schemes within this document can be used for guidance and, in general, should be followed. Lead Examiners, the Assistant Director, and/or the Deputy Director will be consulted for guidance when necessary.

Suspected DUI Cases: Many examinations within the Unit involve antemortem specimens from driving under the influence (DUI) investigations wherein volatiles and/or impaired-driving drugs are suspected to be present. Submitting agency representatives often request that ethanol/drugs be detected, identified, and possibly quantitated. Such analytes within blood samples may be quantitated, but quantitation within urine specimens will be limited to volatile compounds (e.g., ethanol, methanol, acetone, isopropanol). Biological evidence submitted from motor vehicle accident investigations will be treated the same, whether they involve injuries, are from fatalities, or where DUI is simply either suspected or needs to be ruled-out.

Blood-ethanol determinations and/or recreational/prescription drug analyses are usually requested within DUI-related submissions. Drug analyses are typically sought when ethanol is found to be low (or has not been detected at all) from breathalyzer examinations and/or drug usage has been suspected (e.g., DRE evaluations, drug paraphernalia within vehicle found, suspect admissions). Depending on sample matrices, relevant drug-positive findings can be presumptively reported or can be confirmed (and/or quantitated). The search for and/or quantitation of drugs within blood samples will often depend on ethanol results, agency representative requests, and other factors. When breathalyzer refusals have occurred then ethanol and/or drug analyses are sought. In submissions where ethanol findings from samples are high (i.e., equal to or over 0.10 g% blood alcohol content (BAC)), analyses for drugs may be omitted from analytical schemes unless specifically requested. In such cases samples will be saved and preserved accordingly in case future drug analysis requests are received.

Unless sample volumes are limited, samples received from hospitals should be analyzed as if they were never previously analyzed. Data from previous examinations of submitted samples can be evaluated and can influence analytical schemes. Lead examiners (or higher) should be involved in unusual cases or when uncertainty exists in how to proceed during the scheduling of tests.

Because customers often request that ethanol results be reported as soon as possible (e.g., for Per Se hearings at the Department of Motor Vehicles (DMV)), reports containing volatile-only results (or volatile and presumptive drug results) within DUI-related cases can be issued separate from reports that contain drug confirmations and/or quantitations. While drug reports that are issued after ethanol reports are supplemental reports, it is not a requirement that they be titled as such. Tables can be found which help summarize the general analytical schemes for DUI case specimens (see figures). If non-biological evidence is submitted within a DUI investigation (e.g.,

liquid from a bottle seized within a vehicle), the Deputy Director, along with Lead Examiners, will determine which Unit and/or personnel will perform the examinations.

Suspected DFSA cases: Other types of cases within the Toxicology Unit may involve biological evidence from suspected drug-facilitated sexual assault (DFSA)-type investigations. Evidence from these cases will often involve the submission of both urine (recommended) and blood samples. These cases involve analyzing samples for recreational drugs, ethanol, central nervous system depressants (e.g., benzodiazepines, barbiturates), and hallucinogenic compounds. Additional information (e.g., from CT-100 sexual assault kits) may be needed. All DFSA cases will involve confirmatory analytical techniques in addition to any presumptive techniques which may be used. Reports involving presumptive testing-only should not be issued for DFSA-type cases. See below for a generalized analytical scheme. Toxicological analyses will begin within sixty (60) days of the request date for all DSFA-type cases in order to conform to statutes related to sexual assault evidence.

Postmortem cases: Specimens from deceased individuals may be received (e.g., from the Office of the Chief Medical Examiner (OCME) or another entity) and will usually be limited to homicides, motor vehicle accidents, and traumatic suicides (when deemed appropriate by the OCME). Specimens from these cases usually include blood, urine, and/or vitreous humor. If applicable, a memorandum of understanding (MOU) should be followed with regards to case management and regarding which analytes are to be screened, confirmed, and/or quantitated. All confirmations and quantitations not involving drugs/metabolites listed within the MOU should be vetted through a Lead Examiner or higher before allowing additional work to be scheduled or performed.

Other Cases: While the majority of submissions within the DSS laboratory's Toxicology Unit will be from suspected DUI, suspected DFSA, or postmortem type cases, this procedure doesn't preclude other types of cases involving human biological materials to be examined within the Unit (e.g., serum-conversions, proficiency tests).

As new technology is acquired general analytical schemes may change for the different types of cases that are submitted. Some techniques may be substituted, as necessary, and presumptive and confirmatory analyses may be combined into single steps during examinations. While repetition of sample analyses should be done to ensure accuracy, such practices may not always be feasible (e.g., limited sample, time-sensitive cases). Situations where ethanol tests were not conducted or drug quantitations were not performed may be warranted, but must be documented within case files as to why such decisions were made.

6.3.1. Urine

Suspected DUI Cases: Urine samples from suspected driving under the influence (DUI) cases are usually submitted when either breathalyzer results are unavailable or when it is suspected that ethanol may not be the sole contributing factor within the investigation. The analytical scheme for urine samples from suspected DUI cases can involve qualitative/quantitative volatile analyses and qualitative-only analyses for all other drugs. However, if breathalyzer results indicate that little-to-no ethanol played a factor in certain DUI investigations, then the analysis for drugs in those cases may be the only requests that are necessary.

When a submitting agency is unclear as to what may be causing impairment of an individual, the following analytical scheme is often performed: a volatile analysis, a chromatographic/mass spectrometric (GC/MS or LC/MS) analysis, and/or a presumptive screening (e.g., immunoassay). Given certain situations (e.g., when ethanol is identified at blood alcohol-equivalent concentrations equal to or greater than 0.100 g%), drug analyses may not be required and ethanol-only reports may be issued. In such situations documentation within case files will explain why drug requests were not performed. Submitting agencies will have an opportunity to request further [drug] analyses by contacting the DSS laboratory. Information within incoming documents may guide analytical plans. If breathalyzer results are known and indicate that requesting agency representatives only want drug analyses to be performed, then analytical plans can be developed to exclude volatile analyses and only focus on drug analyses. In such situations adequate documentation must exist which explains why certain analyses were (or were not) performed.

If ethanol was determined to be less than 0.1 g% (or not reported at all) within a specimen and no drugs were detected during a screening examination, then further [confirmatory] testing may need to commence before a final report is released. However, if confirmatory-type analyses were performed then no further testing may be necessary. Because of the way Connecticut statutes have been written and because other entities need ethanol results in a timely fashion (e.g., DMV Per Se hearings), reports only indicating quantitative volatile results may be issued separately from drug reports. Quantitation of drugs (other than ethanol) within urine samples will not be done unless prior approval is obtained from a Lead Examiner or higher.

Suspected Drug-Facilitated Sexual Assault (DFSA) Cases: Urine is the recommended type of sample for these cases and should be obtained from the victim as soon as possible. Urine specimens in these cases will be qualitatively tested in order to detect possible past-ingestion of drugs and/or alcohol. For gamma-hydroxybutyric acid (GHB), urine collection times must be less than twelve (12) hours from the suspected drugging/incident. Ethanol should not be

analyzed within urine specimens if urine specimen collection times are greater than forty-eight (48) hours after the incident. Samples that have been collected five (5) days after the alleged incident will not be analyzed without prior approval from either the Lead Examiner, Assistant Director, or the Deputy Director. Quantitation of drugs within urine samples will not be done unless prior approval is obtained from the Lead Examiner or higher.

Postmortem Cases: Urine samples may be submitted and analyzed to supplement findings from other sample matrices (e.g., 6-monoacetylmorphine (6-MAM) for determination of heroin usage). Such samples may also be analyzed for other classes and types of drugs. Additionally in these situations, such urine specimens may be qualitatively analyzed for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine/metabolites, fentanyl/metabolites, methadone/metabolites, opiates, oxycodone/oxymorphone, and phencyclidine (e.g., immunoassay or LC/MS).

6.3.2. Blood

Suspected DUI Cases: While not as frequent as urine, blood samples from DUI-related cases are preferred since such samples tend to give information relating to influence of chemicals at a time close to an occurrence of an incident. Based on the request and based on case history, analyses of these samples can begin with quantitative detection for volatiles. Presumptive screening for drugs-of-abuse [qualitative] can occur. Given certain situations (e.g., when ethanol is identified at concentrations equal to or greater than 0.100 g%), drug analyses may not be required and ethanol-only reports may be issued. In such situations documentation within case files will explain why drug requests were not performed. Submitting agencies will have an opportunity to request further [drug] analyses by contacting the DSS laboratory. If confirmatory-type analyses were performed then no further testing may be necessary. Because of the way Connecticut statutes have been written and because other entities need ethanol results in a timely fashion (e.g., DMV Per Se hearings), reports only indicating quantitative volatile results may be issued separately from drug reports. Typically only toxicologically significant/relevant compounds should be quantitated within blood specimens. Determination of whether compounds should be quantitated can be based on the original request and, if necessary, based on communication with the submitting agency's representative. It may be appropriate to report analytes as qualitative-only or to report them as 'greater than' a certain value (semi-quantitative). Consultation with a Lead Examiner (or higher) will be done when analysts are uncertain whether quantitations should be performed or how reports should list results.

Suspected Drug-Facilitated Sexual Assault (DFSA) Cases: These cases may call for screenings of blood for recreational drugs, ethanol, and other central nervous system depressants (e.g., benzodiazepines, barbiturates). Targeted screens for certain DFSA drugs (e.g., GHB, flunitrazepam) may also be necessary. Relevant drugs within blood specimens are quantitated based on the original request and, if need be, based on further communication with the submitting agency's representative. Specimens should not be analyzed for GHB if their collection times are greater than twelve (12) hours after the time of drugging/incident and ethanol should not be analyzed within specimens if their collection times are greater than twenty-four (24) hours after the incident unless approval from a Lead Examiner or higher has been obtained. Urine samples that have been collected five (5) days after the alleged incident and blood samples that have been collected after two (2) days will not usually be analyzed. Blood may be tested if more than twenty-four (24) hours lapse between the time of incident and the collection time in order to be able to better interpret the significance of positive urine findings (or if target drugs are known to have longer half-lives). Because DFSA-type cases can be unusual and non-routine, consultation with a Lead Examiner or higher should be considered when determining which tests be included during the development of an analytical plan or scheme.

Postmortem Cases: When death is delayed after an incident, antemortem blood evidence is often preferred because results from these specimens can give information prior to post-hospital intervention. Typically only limited drugs/metabolites will be sought within evidence from these cases (i.e., OCME according to the MOU). Determination of whether compounds should be quantitated can be based on the MOU or communication with the submitting agency's representative. It may be appropriate to report analytes as qualitative-only or to report them as 'greater than' a certain value (semi-quantitative). Consultation with a Lead Examiner (or higher) will be done when analysts are uncertain whether quantitations should be performed or how reports should list results.

6.3.3. Vitreous Humor/Fluid:

Postmortem Cases: Vitreous fluid samples may be submitted and analyzed to supplement findings from other sample matrices. When positive ethanol findings within blood specimens occur, confirmation of ethanol will be achieved through the quantitative analyses of associated vitreous fluid specimens. When morphine is identified in a blood sample and there is no associated urine sample submitted with the case, the vitreous sample will be qualitatively analyzed for 6-acetylmorphine (aka: 6-monoacetylmorphine (6-MAM)), to indicate possible heroin use.

6.4. Analytical Schemes for Screening Tests

Analytical schemes can begin with the use of screening protocols for drug class determination. Toxicological screening techniques can include, but are not limited to, immunoassays, extractions, and/or headspace analyses using selective detectors on gas chromatographs. A list of analytes that can be screened within the Toxicology Unit using immunoassay and/or headspace gas chromatography can be seen in Table 1. Additional analytes may be added, as necessary.

Table 1: Analytes which can be Routinely Screened for within DUI Cases

Ethanol	Benzodiazepines	Cocaine	Oxycodone
Amphetamines	Buprenorphine	Methadone	Phencyclidine (PCP)
Barbiturates	Cannabinoids (Δ^9 -THC)	Opioids	

Alternatively, confirmatory techniques (e.g., gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS)), or similar instrumentation can be used to screen samples for drugs/metabolites. When agreed upon by a submitting agency, analyses may be limited to only select analytes (e.g., OCME's MOU). Table 2 lists drugs/metabolites which have been agreed, based on an MOU, to be commonly screened during the analysis of OCME samples. Even though LC/MS is a confirmatory technique, certain experiments may be used within the instrument which allow it to rapidly examine specimens and thus be considered a screening tool. When a confirmatory technique is used in lieu of a screening technique, and the experiment contains sufficient information to qualitatively identify compound(s), analysis of a second aliquot for confirmation purposes is not required. If necessary, the Lead Examiner or higher should be consulted to determine if further confirmatory testing is needed.

The purpose of screening techniques, especially in DUI cases, is to help rule-out the possible presence of analytes at certain detectable limits. When screening results are negative it indicates that certain analytes/classes of analytes are not detected. When screening results are positive it indicates that certain analytes/classes of analytes are present. Depending on the screening technique, further testing may be warranted. Screening, sometimes referred to as presumptive testing, should have minimum detection limits for analytes of interest that include therapeutic concentration ranges for drugs. The selection of the screening technique(s) usually depends upon the case type, the amount of available specimen, and what questions need to be answered for the submitting agency.

There are some situations wherein urine samples may only be analyzed by a screening technique and a report will be issued listing presumptive results. In those situations, when presumptively positive, a second aliquot of urine can be analyzed to ensure

accuracy in the findings. Presumptive-positive drug results (e.g., from immunoassay data) within urine samples may be reported without confirmatory analyses being performed. In those situations a qualifying statement will accompany the report advising the reader that such results were only presumptive.

6.5. Analytical Schemes for Confirmatory Tests (see figures)

In the case of LC/MS and GC/MS, chromatography and mass spectrometry are considered two orthogonal techniques. Additionally, a different MS experiment may also be considered an orthogonal technique (e.g., full scan versus MS/MS versus selected ion monitoring (SIM)). Often, confirmatory tests for targeted analytes are more specific than screening assays which are used for initial detection. All confirmatory tests will include the use of positive and negative controls for the analyte(s) of interest. Positive control(s), however, need not be analyzed within the same batch/sequence as unknown samples as long as the positive control(s) are analyzed under the same analytical conditions and conform to quality control criteria found within specific experimental methods. When a screening technique indicates the possible presence of an analyte in a specimen (e.g., urine), confirmation of the identity of the analyte in a second aliquot from the same specimen is acceptable. Whenever possible and practical, the use of mass spectrometry is recommended as a confirmatory technique.

Table 2: Analytes Routinely Screened and/or Quantitated within Postmortem Cases

11-Hydroxy Delta-9 THC	Desalkylflurazepam	Methylephedrine
6-Monoacetylmorphine _(free)	Diazepam	Midazolam
Acetone	Dihydrocodeine / Hydrocodol _(free)	Morphine _(free)
Alpha-Hydroxyalprazolam	Diphenhydramine*	Norbuprenorphine _(free)
Alprazolam	EDDP	Nordiazepam
7-Aminoclonazepam	Ephedrine/Pseudoephedrine	Norfentanyl
Amobarbital	Estazolam	Norpropoxyphene
Amphetamine	Etizolam	Oxazepam
Barbiturates	Fentanyl	Oxycodone _(free)
Benzoyllecgonine	Flurazepam	Oxymorphone _(free)
Blood Alcohol Concentration (BAC)	Hydrocodone _(free)	Pentobarbital
Buprenorphine _(free)	Hydromorphone _(free)	Phencyclidine
Butabarbital	Hydroxyethylflurazepam	Phendimetrazine
Butalbital	Hydroxytriazolam	Phenmetrazine
Cannabinoids	Isopropanol	Phenobarbital
Chlordiazepoxide	Lorazepam	Phentermine
Clobazam	MDA	Phenylpropanolamine
Clonazepam	MDMA	Propoxyphene

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Cocaethylene	MDEA	Secobarbital
Cocaine	Methadone	Selegiline
Codeine _(free)	Methamphetamine	Temazepam
Delta-9 Carboxy THC	Methanol	Triazolam
Delta-9 THC	Methorphan (Dextro/Levo)*	Zolpidem

* May only be detected when using LC/MS (detection by immunoassay dependent on concentration)

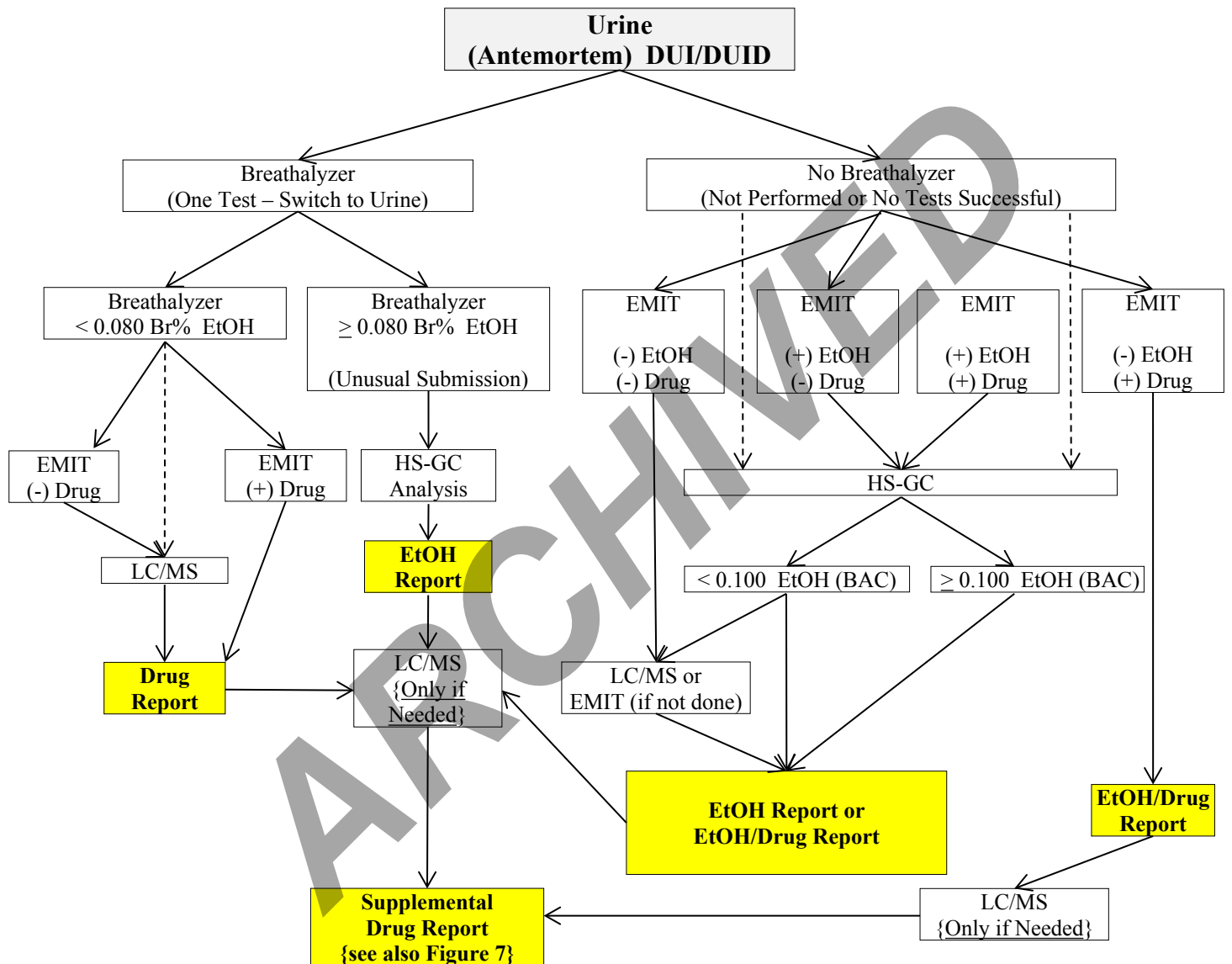
When urine samples are positive for volatiles after presumptive testing is done (i.e., EMIT), then confirmatory testing for volatiles is usually performed. Depending on the situation and the amount of volatiles (e.g., ethanol) found within the specimen, further analyses for volatiles/drugs may be performed. Documentation must be present which explains decisions as to why the analysis of certain analytes was excluded.

While evidence from sexual assault cases may initially be analyzed by presumptive techniques, confirmatory techniques are usually used for these cases, when applicable. In cases where the presumptive technique also provides confirmatory results (e.g., accurate mass-LC/MS) additional [confirmatory] analyses may not be required. Limited sample or other situations may influence the number of tests that will be applied to certain evidence. Good laboratory practice and sound judgement will be used by analysts when evaluating and deciding on analytical plans. The Lead Examiner (or higher) will be notified of any unusual situations or when potential problems exist with analytical schemes.

6.6. Analytical Schemes for Quantitations (see figures)

Quantitation is performed on analytes based on importance to a case (as determined by case history, specimen volume, specimen type, and toxicological significance) and based on agreements with submitting agencies (e.g., MOU with OCME). Appropriate internal standards (e.g., deuterated) and controls (positive/negative) are used within each batch/sequence. Calibrator solutions (i.e., calibrators) need not be analyzed within the same batch/sequence as unknown samples as long as the control(s) are analyzed under the same analytical conditions and conform to quality control criteria found within specific experimental methods. A lead Examiner (or higher) should be consulted if utilizing calibration data for quantitations wherein such data is greater than thirty (30) days.

Figure 1: Analytical Scheme for DUI Antemortem Cases (Urine)



Note-01: The term HS-GC throughout this document can mean either HS-GC(FID) or HS-GC/MS(FID)

Figure 2: Analytical Scheme for DUI Antemortem Cases (Blood)

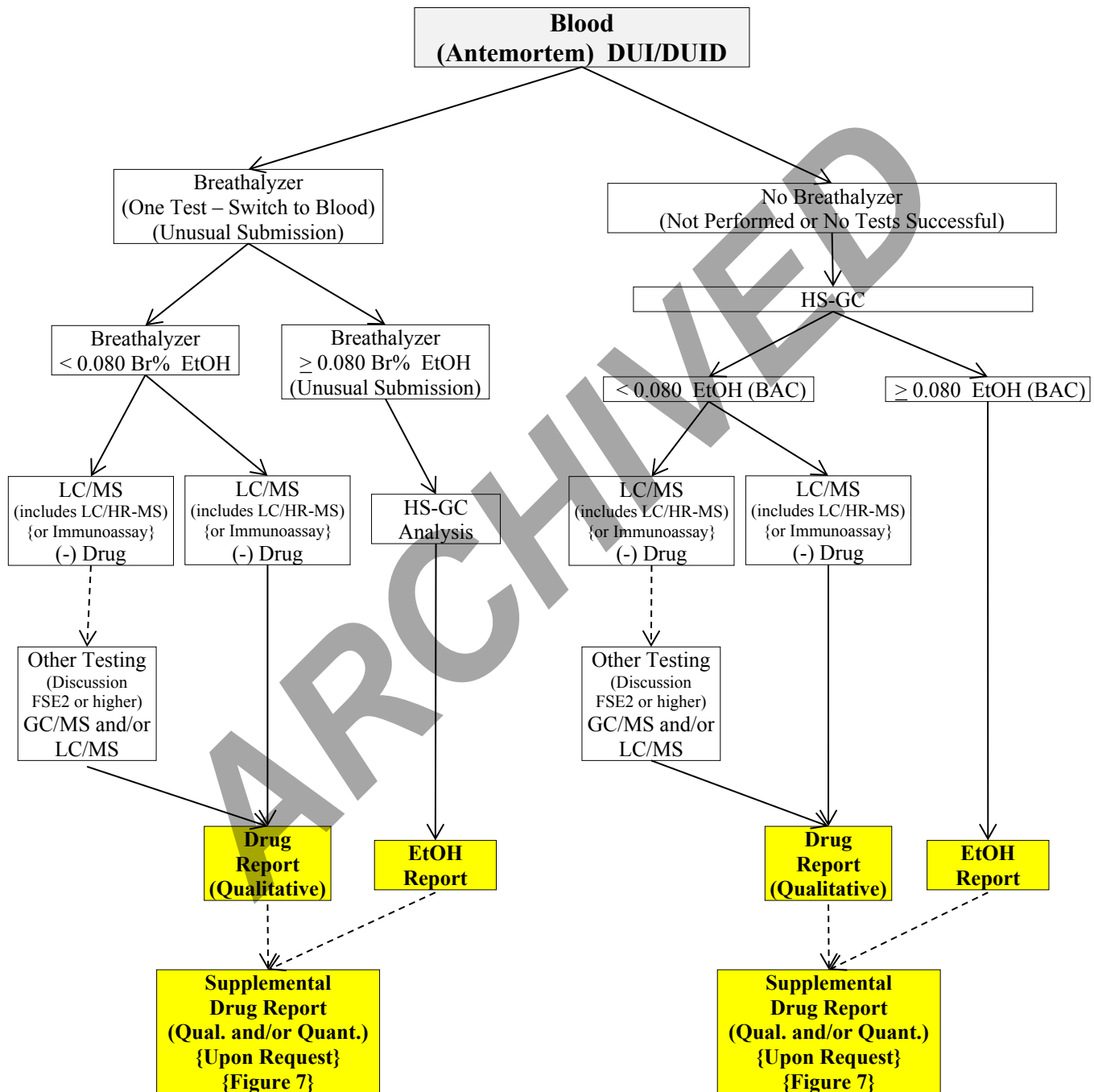
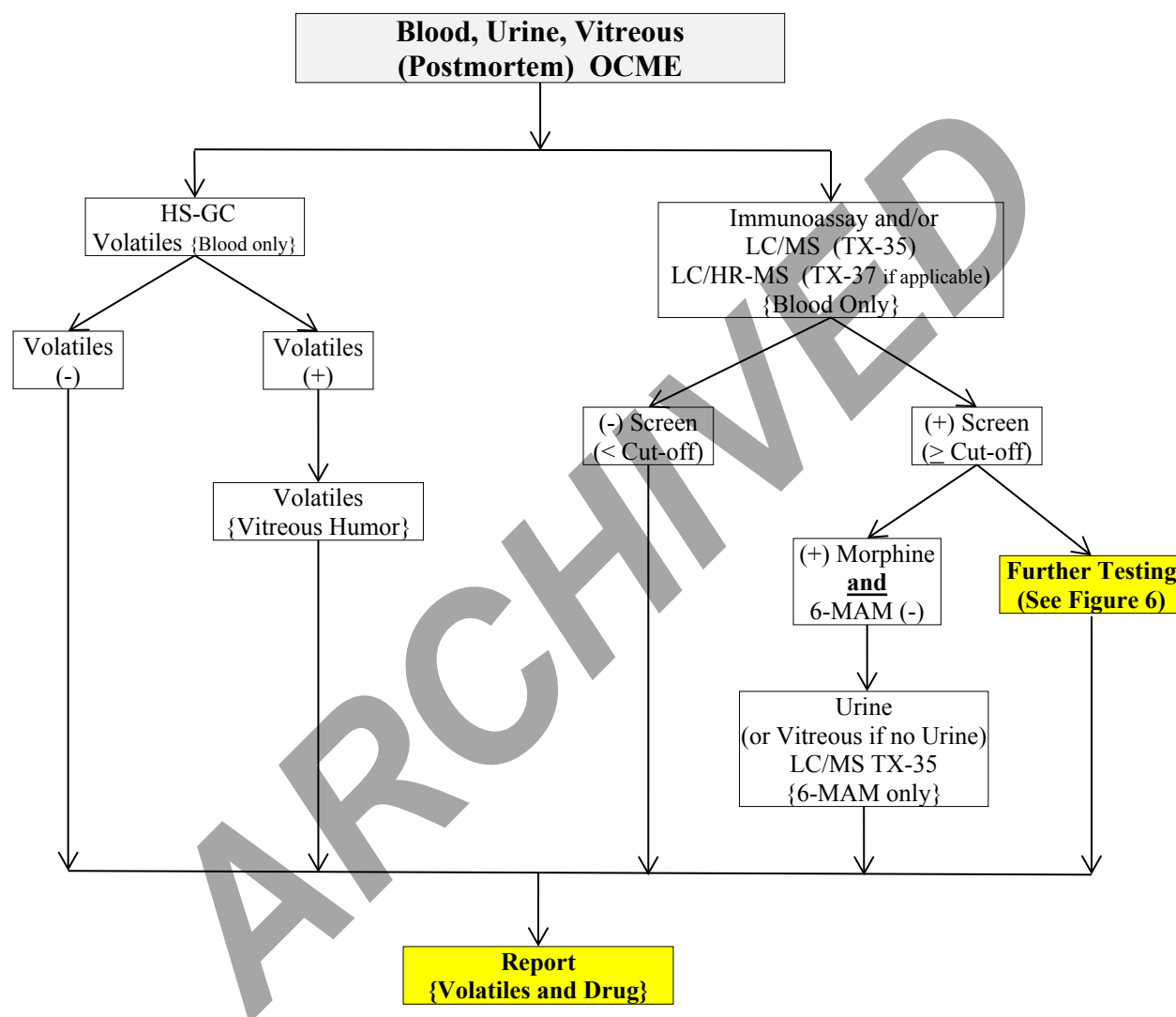


Figure 4: Analytical Scheme for Postmortem Cases (Blood, Urine, Vitreous Fluid)



Note-02: Variations in this (and other) schemes are allowed depending on sample volume, customer requests, and other reasons deemed toxicologically applicable. Such variations will be recorded in appropriate case file and/or batch paperwork.

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Figure 5: Analytical Scheme for Antemortem Cases (Supplemental/Confirmation – Urine)

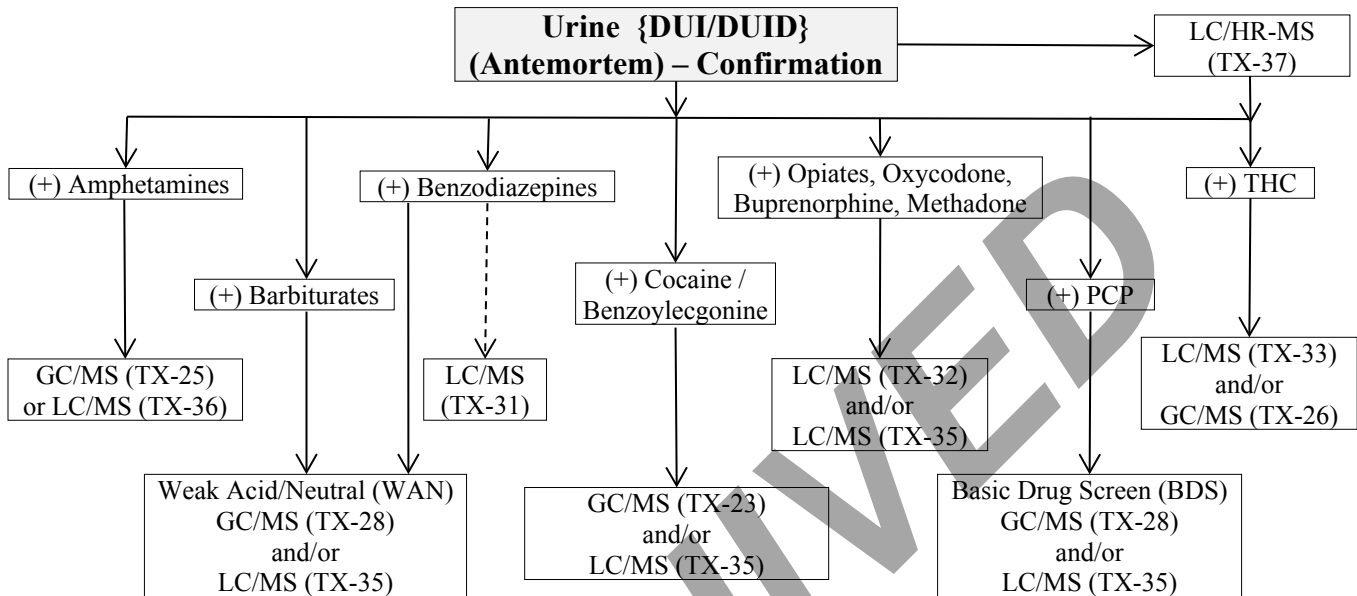


Figure 6: Analytical Scheme for Postmortem Cases (Further Testing Confirmation – Blood)

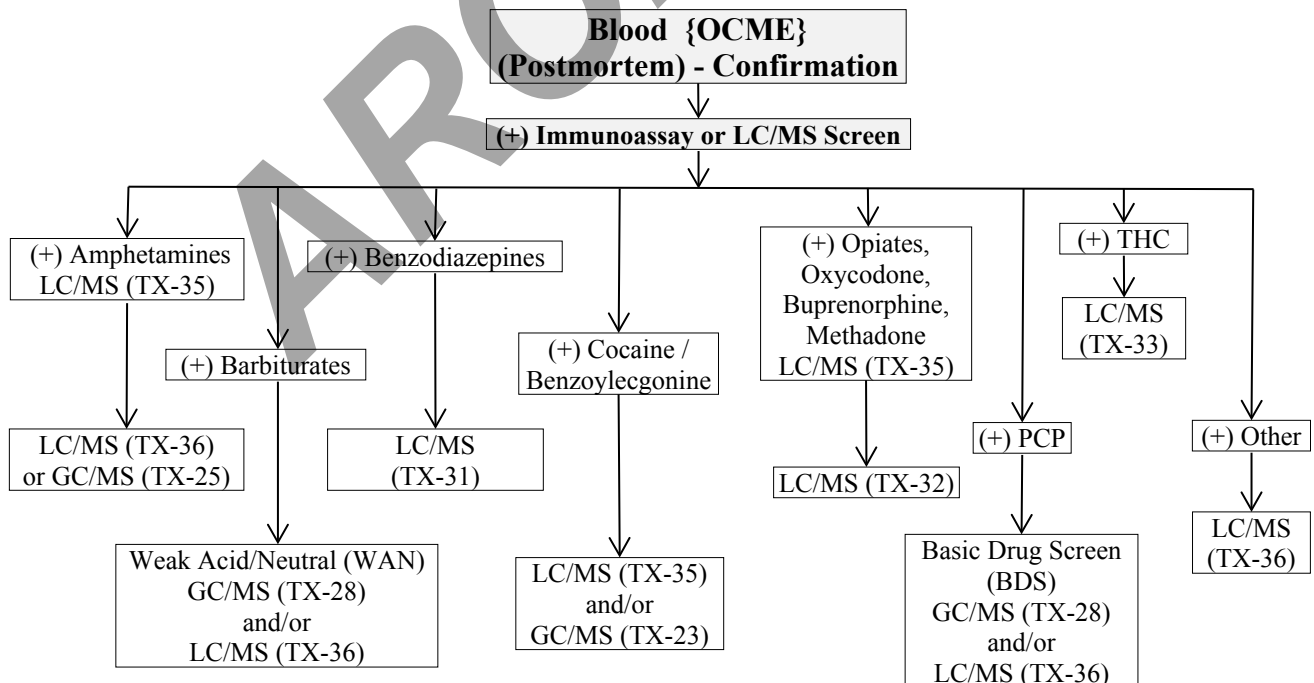
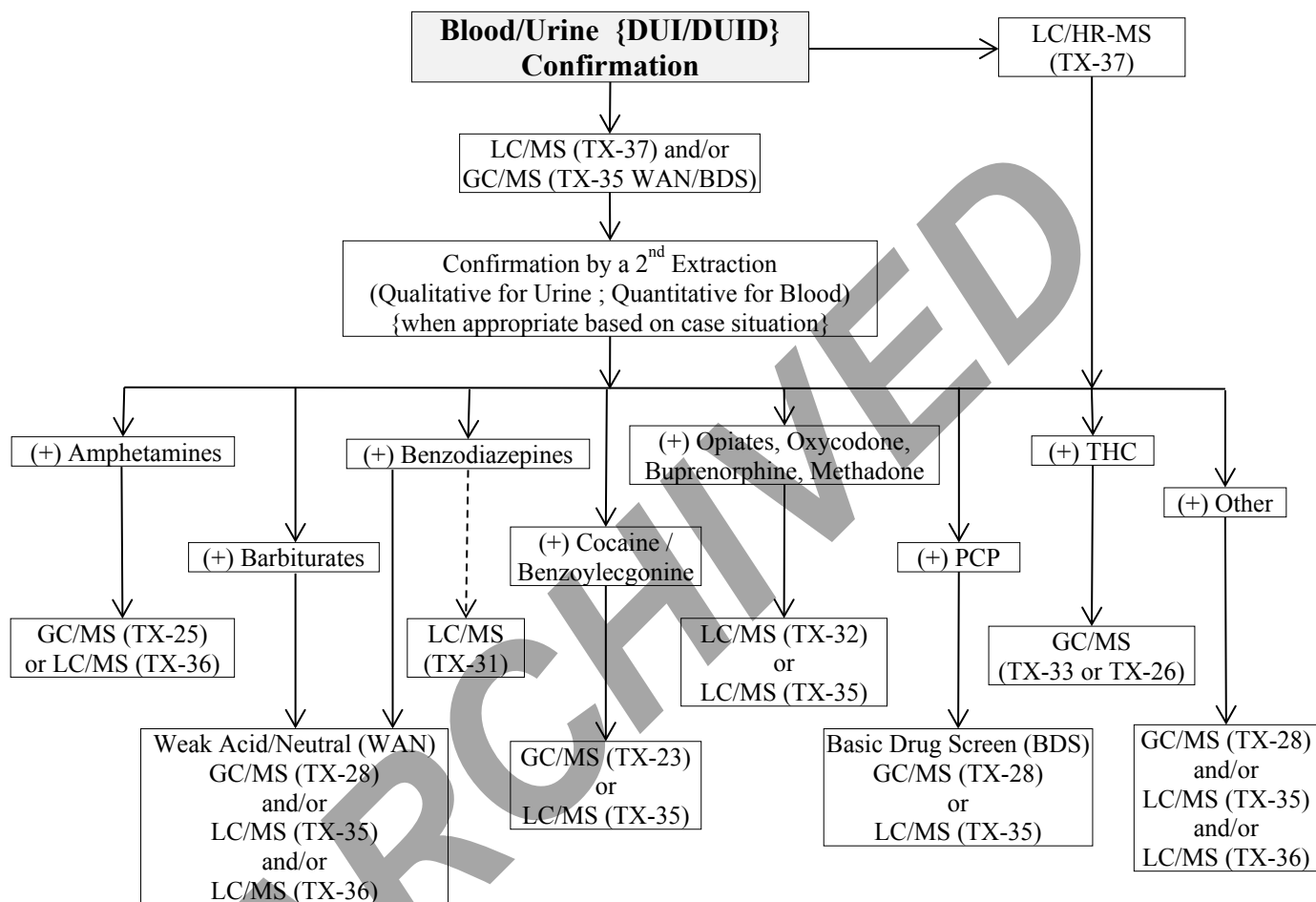


Figure 7: Analytical Scheme for DUI Antemortem Cases (Confirmation – Blood/Urine)



7. Uncertainty

Procedural uncertainty is reported with all quantitative results and should be evaluated annually. The overall uncertainties can be calculated based on an evaluation of control performance (variance). Documentation of the calculation of uncertainty can be maintained in a notebook in the laboratory. Included in this notebook should be documentation (or appropriate references) of reference materials and equipment utilized in the procedure that could affect the quantitative accuracy of the method. 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 can be reported based on the response of batch controls.

8. Reagent Validation

When a new lot of a solvent or reagent is needed they will be appropriately validated prior to use. Analysts will verify that reagents/solutions worked as expected and that there were no interfering or unexpected results observed. Documentation for the acceptability will be appropriately retained (e.g., within batch documentation, logbook). A solution is considered validated and acceptable for use when the solution is shown to have worked in the procedure, and blanks/negative controls are shown not to contain contaminants that will interfere with the overall findings. Documentation should include recording the validation of the reagent on the batch runsheet and having the batch reviewed. Completing the Reagent Logbook, includes stating the name of the reagent/solution, lot number, the date made by who, and the date it was validated. If created in-house, the lot number should include information related to date made and preparer's initials (e.g., MPR07212016). If the same solution is made using different reagents or at different times during the same day, lot numbers can be differentiated using hyphenated letters (e.g., JJ07042016-a, JJ07042016-b). Solutions may be marked with a colored sticker (e.g., green) according to which method the solution is validated for, the date validated (the batch date), and the analyst's initials. For materials that are purchased in multi-bottle lots, once one bottle from the lot is found to be acceptable for a particular use, all the bottles in the lot can also be marked as validated for that use (or related use, as appropriate). Bottles with working solutions will be labeled with appropriate information (e.g., solution name, lot number (if generated in-house will include data and initials of preparer), expiration date (if applicable and if different than the standard one year from date of validation), initials (if different from in-house lot number), colored label (e.g., green) with validation information, applicable safety information. When applicable, quality control solutions (e.g., controls, calibrators) should be purchased. If solutions need to be made in-house, good quality assurance practices will be employed (e.g., varying reagent lot numbers, varying calibrator solutions, recording trend lines, verifying newly prepared solutions against older solutions)

9. Abbreviations

The following abbreviations may be found in the procedures and case notes within the Toxicology Unit:

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

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GC MS	Gas Chromatography Mass Spectroscopy
IR	Infrared
UV	Ultraviolet
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	Cocaine
BE	Benzoylcegonine
Op	Opioids
THC	Delta-9-Tetrahydrocannabinol
COOH-THC	Carboxy-delta-9-tetrahydrocannabinol
THC-OH	11-Hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC, OH-THC)
Benzo	Benzodiazepine
SMA	Sympathomimetic Amines
GHB	Gamma-hydroxybutyrate or gamma-hydroxybutyric acid
OCME	Office of the Chief Medical Examiner
MOU	Memorandum of Understanding
Volatiles	Methanol, Ethanol, Acetone, Isopropanol

10. References

DSS Quality Manual

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

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Handbook of Analytical Toxicology, Cravey, R. and Baselt, R. Eds., Biochemical Publications: Davis, CA, 1981

Poison Detection in Human Organs, 4th Ed., Curry, A., Ed., Charles C., Thomas: Springfield, IL, 1988

Principle of Forensic Toxicology, 2nd ed., Levine, B. Ed, American Association of Clinical Chemistry, Washington, DC, 2003.

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History

- 3 Updated verbiage and formatting within the entire document. Inserted figures and tables to summarize schemes. Inserted information about OCME samples. Removed unnecessary verbiage throughout document. Added schematic figures to summarize methodology. Added History section.
- 4 Updated verbiage, grammar, and formatting within the entire document. Added 'Assistant Director' throughout document. Updated to allow for either LC/MS Screening or Immunoassay techniques as an initial screening tool. Clarified that the use of LC/MS as a screening tool may not require further analysis. Updated flow charts to include LC/MS Screen and other LC/MS Quantitative methods. Removed bias from consideration of Uncertainty. Added limitation statement within Table 2. Updated section 6.5 and the figures.
- 5 General unknown drug screen of comprehensive drug screen added. Discussion about postmortem cases added. Wording added to allow the extraction process and instrumentation analysis to be performed by separate analysts. Added abbreviations PM and OCME. Definition for work product expanded. Definitions for basic drugs of abuse screen and comprehensive drug screen added. Updated verbiage and formatting within the entire document. Inserted figures and tables to summarize schemes. Inserted information about OCME samples. Removed unnecessary verbiage throughout document. Added schematic figures to summarize methodology.
- 6 Fixed grammar and spelling errors throughout document. Assistant Director position added throughout document. Added statement about LC/MS analyses in paragraph 1 of section 6.3.1. Changed wording in paragraph 1 of section 6.3.1. Added "other than ethanol" to statement in last sentence of paragraph 1 of section 6.3.1. Changed "customer" to "submitting agency's representative" in paragraph 2 of section 6.3.2. Updated postmortem cases section under suction 6.3.2. Updated Section 6.4 and added paragraph about situations in which urine samples may only be analyzed by a screening technique. Added a brief statement under Table 2 in Section 6.5. Added LC/MS to Figures 1, 2, and 3. Updated Figure 4 to combine immunoassay and LC/MS screen. Added LC/MS to figures 5 and 6. Changed THC confirmations within figures 5

and 6. Updated Figure 7 to include LC/MS. Updated format and added MOU abbreviation in section 9.

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Updated itemization numbers to reflect new format change. Updated analytical plans for DUI cases. Updated wording and other material within sections 5 and 6.1. Information regarding expiration dates added in section 6.1. Changed JT to LIMS-Plus throughout document. Updated wording in Section 6.1 for clarity. Added use of tamper-evident devices within section 6.2. Moved paragraph about case history from section about postmortem cases in section 6.3 and moved to section 6.3. Added information under suspected DUI cases in sections 6.3, 6.3.1, and 6.3.2. Updated sections 6.3 and 6.4 regarding minimum detection limits for analytes of interest, including therapeutic ranges. Added statement about documentation to section 6.5 and added the ability to use older calibration data for quantitations. Updated figures throughout document.