Las Vegas Metropolitan Police Department
Forensic Laboratory
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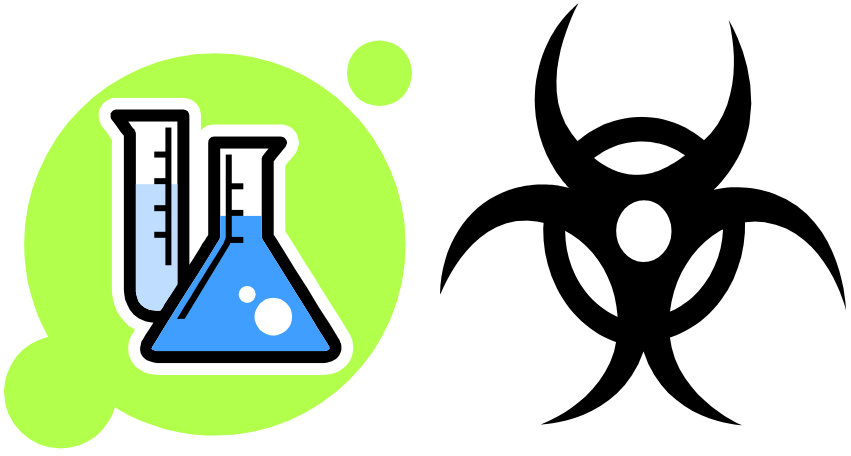
Toxicology Technical Manual



0.0 Title: TABLE OF CONTENTS

NOTE: Hyperlinks were accurate at the time of manual publication.

LVMPD FORENSIC LABORATORY TECHNICAL PROCEDURES TOXICOLOGY

1.0 Title: INTRODUCTION

The following drug identification analytical techniques are offered as the recommended procedures currently available with the Las Vegas Metropolitan Police Department (LVMPD) Forensic Laboratory's Toxicology Detail. This manual was drafted with input and comment from the Forensic Scientists and managers of this laboratory system. In that regard it meets the goal of providing the Laboratory with a workable guideline encompassing established facts, principles, and theories widely accepted by the general scientific community. The intent is to respond to the needs of the profession, the investigative agencies, the courts, and ultimately, the citizens they serve.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

2.0 Title: EVIDENCE HANDLING AND WORKFLOW

The following details the handling of evidence and workflow in the Toxicology Detail of the LVMPD Forensic Laboratory:

2.1 Evidence Handling

2.1.1 Evidence Receipt

Analysis will only be completed on human antemortem samples. Post-mortem samples will be returned to the requesting agency.

Evidence received from the Las Vegas Metropolitan Police Department Evidence Vault and/or authorized drop locations will be tracked and handled using the policies and procedures under section 7.4, Handling of Evidence (Test Items), in the LVMPD Forensic Laboratory Quality Manual. After evidence has been data entered into the evidence management system it will be stored in a refrigerator in the Toxicology Lab or in the Forensic Laboratory Evidence Vault unless otherwise indicated.

2.1.2 Evidence Storage

Biological evidence will be stored in a refrigerator in the Toxicology Lab when samples are not in the process of being analyzed unless otherwise indicated.

2.1.3 Evidence Return or Transfer

Upon completion of analysis, the evidence will be resealed with evidence tape, the tape will be initialed and dated, and the chain of custody on the evidence will be signed. A move to the Toxicology refrigerator location in the evidence management system is required; the evidence is then sent to the LVMPD Evidence Vault or retained for additional testing. NHP evidence will be returned to the NHP.

2.1.4 Requests for Reanalysis by an External Laboratory

Court orders from the Defense for reanalysis by an external laboratory should be forwarded to the Quality Detail. The Quality Detail will verify that the court order contains the necessary information needed to release the evidence for reanalysis. The Quality Detail will then forward the court order to the Toxicology Manager/Supervisor who will ensure that all work has been completed on the case prior to the release of evidence. If further analysis is pending upon request, the Toxicology Manager/Supervisor should contact the requesting agency and/or Prosecutor's office to inquire if the pending analysis should be completed prior to the release of evidence. The conversation should be documented in the case file. When the evidence is released directly to the Defense or their agent and sent to a laboratory of

their choice, the evidence will be considered unsuitable for reanalysis by the Toxicology Detail upon its return to the Evidence Vault. See Forensic Laboratory Quality Manual Appendix P for further information.

2.2 Evidence

2.2.1 Evidence Description

Blood Kit

A standard blood kit is a white approximately 5" x 3" x 1 ½ " cardboard box. Formatted with blue print. The box contains two (2) 10 mL gray top test tubes (containing sodium fluoride/potassium oxalate) secured in a foam holder.

Urine Kit

A standard urine kit is an approximately 3" x 3" x 3" white cardboard box. The box contains a wide-mouth plastic urine specimen bottle with cap, sized to fit in the box. Wide-mouth plastic urine sample bottles with caps may also be packaged in either plastic or paper bags.

Evidence received in other forms will be described in case notes and on the Laboratory Report of Examination (see section 2.9 Reporting).

2.2.2 Case Information

The analyst will defer to the information on the outside of the blood or urine kit, rather than the request form, for event number, subject name, incident time, incident, etc.

The subject's first and last name as it appears on the front of the blood or urine kit should match the name on the evidence management system label. This will be used as the name on the Forensic Laboratory Report of Examination. The first analyst that receives the kit will interpret the name. Subsequent analysts will defer to that interpretation for their report.

If the name on the blood tubes is grossly different than the name on the blood kit, the Forensic Laboratory Report of Examination will reflect the name per blood kit, name per blood tubes. Middle initials and suffixes are typically not entered into the evidence management system, but if they appear on the evidence management system label and match the information on the blood kit they can be used on the Forensic Laboratory Report of Examination; the name in the evidence management system does not need to be amended to exclude this information.

2.2.3 Blood Tubes

When a standard blood kit is received, it is recommended to use the tube with the most blood for analysis. When evidence is received in forms other than a standard blood kit (e.g., tubes collected at a hospital), the following tubes may be used:

Gray top (sodium fluoride, potassium oxalate)
Lavender top (EDTA)

Pink top (EDTA)
Green top (heparin)
Red top (no preservative)

Whole blood that is clotted but received in the above test tubes may be homogenized prior to use. Serum and plasma, as well as blood collected in light blue top tubes (sodium citrate), serum separator tubes (tiger top), or any other tubes which causes the blood to clot, are not suitable for testing. These samples may be sent to an approved outside laboratory for testing if mitigating circumstances apply.

2.2.4 Sample Suitability

When a sample is determined to be unsuitable for testing (e.g., insufficient sample volume, serum rather than whole blood, etc.) the requesting agency will be notified before cancelling testing. Testing is cancelled only when none of the requested work can be completed. For example, if there is insufficient sample volume to complete a drug screen, testing is cancelled. By contrast, if a sample screens positive for two drugs and one of the drugs has been confirmed but there is insufficient volume to complete the second drug confirmation, testing is considered complete.

2.2.5 Consumption of Evidence

Unnecessary consumption of the sample shall be avoided, but it is occasionally necessary to consume a sample in order to complete the analysis properly. The analyst will document in their case notes and on the Laboratory Report of Examination when the entire sample is consumed.

2.3 Workflow

Casework samples should follow the workflow:
blood alcohol (when requested) => drug screen (when requested) => confirmation (if necessary).

Cases are prioritized in the following order:

1) Cases with a court deadline
2) Felony cases
3) Cases in which public safety is an issue (e.g., suspect has multiple DUI incidents in a short time span awaiting analysis)
4) Routine misdemeanor cases

Workflow Exceptions

- If no drug screen was requested but the blood alcohol result is less than 0.085 g/100 mL, a standard drug screen will be completed.

- If no alcohol analysis was requested but the case is negative for drugs, alcohol analysis will be completed if there is no valid evidential breath test on record.

- The coadministration of cocaine and ethanol produces cocaethylene. If no alcohol analysis was requested but the case confirms for cocaethylene above the cutoff, alcohol analysis will be completed if a valid evidential breath test was not given.

  Note: The analyst will defer to the information on the Toxicology Request Form to determine if a valid breath test was given. If no information is available on the form, the Breath Alcohol Unit will check breath records for a valid test.

2.3.1 Casework Samples – Alcohol

Casework samples requested for alcohol analysis will be analyzed according to Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace.

Blood is the preferred matrix for alcohol testing. When both blood and urine are received for the same case, the urine will only be analyzed if the blood is not suitable for testing.

2.3.2 Casework Samples – Drug Screening

Casework samples requested for drug analysis will first be analyzed using a screening technique. Blood samples will be analyzed according to Chapter 3.0 ELISA Blood Screening Procedures. Urine samples will be sent to an outside laboratory for analysis.

Some cases result in more than one blood alcohol kit being drawn with the same event number and different blood draw times. These types of cases are referred to as multiple draw cases. When a multiple draw case requires drug screening, only the first draw will be used. The second draw may be used if there is insufficient sample in the first draw or other extenuating circumstances exist.

Confirmation of drugs will be performed on samples which have positive screening results and/or on samples which the submitting officer requests a confirmation of a drug which the Forensic Laboratory does not have a screening technique.

To assist with the confirmation workflow, it is recommended to note on the screening worksheet when the volume of blood in both tubes is less than 4 mL and target drugs that are confirmed in-house.

In most cases, samples received in sexual assault cases will be sent to an outside laboratory for testing. The Toxicology Manager or designee will review all requests involving sexual assaults to make the determination.

2.3.3 Casework Samples – Confirmation

Casework samples needing confirmation analysis will be analyzed according to the procedures outlined in Chapters 4.0 Confirmation Testing and 4.1 (procedures for blood).

If sample volume is low, the preferred order of confirmation analysis is:

- For all incidents except Under the Influence of a Controlled Substance:
    1) Illicit drugs/metabolites as defined in NRS 484C.110
    2) Drugs/metabolites not listed in NRS 484C.110

- For Under the Influence of a Controlled Substance incidents, cannabinoids should be confirmed after other controlled substances listed in NAC 453 unless it is known that the subject is under the age of 21.

2.3.4 Outsourcing

Pending Manager or designee approval, casework and Department samples may be sent to an approved outside laboratory to be analyzed for substances for which LVMPD does not have a validated method. These substances may be listed on the request form as drugs suspected. Routinely, only samples from felony cases will be outsourced when all in-house results are negative. If some analysis has been completed in-house prior to outsourcing, a Technical Review will be completed prior to the outsourcing.

2.3.5 Subcontracting

Under special circumstances, casework and Department samples may be sent to an approved outside laboratory to be analyzed for substances for which LVMPD does have a validated method. Toxicology Manager or designee will determine when subcontracting may occur. If some analysis has been completed in-house prior to subcontracting, a Technical Review will be completed prior to the subcontracting.

2.4 Dates of Testing

"Start date of testing" is defined as the date the analyst transfers the evidence into their custody in the LIMS. "End date of testing" is defined as the date the analyst has completed all activities of the analysis, including data review and kit sealing. In most cases this date will be the date the analyst transfers the evidence out of their custody in LIMS. If the nature of testing is such that the analyst must pass the evidence to another analyst prior to completion of testing (e.g., a rush case where results are needed in several days), the analyst will document the "end date of testing" in their case notes.

In the event that the analyst did not transfer the evidence into their custody in the LIMS prior to testing (e.g., the LIMS is unavailable), the analyst will document the "start date of testing" in their case notes.

The dates of testing will be included on the report.

2.5 LIMS

The LIMS utilizes an external application called FA Batch Processing for running samples in batches. After a batch has run, the data is imported from the instrument to the Batching module and then imported into the corresponding worksheet in LIMS. Data entry by the analyst may be necessary in certain circumstances. In the event that the LIMS or LIMS Batching is unavailable, samples may be run outside of the LIMS and the results hand-entered when the system becomes available. The paperwork generated by running the samples outside of the LIMS shall then be uploaded into the Object Repository with the exception of QC packets, which will be stored in Qualtrax.

2.5.1 LIMS Naming Convention

When FA Batch Processing is used for blood alcohol and drug confirmation, the number generated equates to Lab Number – unit record<space> item. For example 14-12345-2 1 indicates the second unit record for the first item of evidence for Lab Number 14-12345.

2.6 QC Packets

2.6.1 QC Packet Naming

QC Packets should be named in the following manner:
<Procedure> QC Packet <Date (MMDDYY)> <Instrument (for blood alcohol and confirmations only)> <Analyst's Initials>

For example:

- BA QC Packet 041017 GC#5 DK
- ELISA QC Packet 041017 NO
- THCB QC Packet 041017 GCMS#9 SW

QC packets for quality control checks (no casework samples) should follow the above naming convention with the addition of "QC".

For example:

- BA QC Packet 041017 GC#5 DK - QC

2.6.2 QC Packet Contents

QC Packets will contain at least:

- load list/sequence
- calibration data
- positive / negative controls
- lot numbers with corresponding expiration dates

See Chapters 3.0 ELISA Blood Screening for additional Drug Screening QC Packet content.

Hardcopies of documents (e.g., lot number sheets, load lists, etc.) that are integrated into an electronic QC Packet must be initialed by the analyst. Page numbers are not required.

2.6.3 QC Packet Storage

QC Packets for casework and Department samples will be stored in Qualtrax.

2.7 Documentation of Rejected Data

If data is not used, the reason, the identity of the individual(s) taking the action and the date shall be recorded in the case file.

2.8 Notes and Corrections

All notes, additions, and corrections, including those on worksheets, will be initialed and dated.

2.9 Reporting

2.9.1 Evidence Description

A description of the evidence will appear on the Laboratory Report of Examination.

2.9.1.1 Blood

When a standard blood kit (as defined in section 2.2.1) is received the analyst will add this statement to the report:

- "That each blood kit received was a standard blood kit containing two gray top tubes of whole blood. Only one blood tube per kit was used for analysis;"

Blood evidence received in forms other than a standard blood kit will be described in detail in a statement on the report. If more than one blood tube contained blood it will be noted which blood tube was used for analysis. For example:

- "That an envelope containing one lavender top tube, one green top tube, and one blue top tube was received. The lavender top tube was used for analysis;"
- "That a blood kit containing one gray top tube of whole blood and one empty gray top tube was received;"

If it is necessary for an analyst to use more than one test tube to complete the requested analyses, the analyst will describe which tubes were used for the analyses. For example:

- "That a standard blood kit containing two gray top tubes of whole blood was received. The first blood tube was used for the drug screen, cocaine and cannabinoids confirmation. The second blood tube was used for benzodiazepines confirmation;"

2.9.1.2 Urine

When a standard urine kit (defined in section 2.2.1) is received the analyst will add this statement to the report:

- "That a standard urine kit containing urine in a wide-mouth plastic urine specimen bottle with cap was received;"

Urine evidence received in forms other than a standard urine kit will be described in detail in a statement on the report.
For example:

- "That a metal can containing a plastic conical tube of urine was received;"

2.9.2 When No Conclusion Can Be Reached

Occasionally circumstances are such that no result can be obtained for a sample. Some such circumstances are listed below followed by the statements that should be used on the Laboratory Report of Examination:

- Quantity of blood is not sufficient to complete the test – "further analysis required but not performed due to insufficient sample quantity"
- Substance interferes with analyte of interest – "unable to determine due to interference"

2.9.3 When Further Analysis Is Needed

Occasionally there is not time to complete all confirmation analyses prior to a court date. The following verbiage should be used on the report for those analytes that will be completed subsequently:

- "Further analysis pending."

A supplemental report will then be issued with the subsequent results. The supplemental report will contain a statement referencing the analyst, distribution date, and results of the original report as in the example below. This statement should be listed below the confirmation results table on the supplemental report.

- See Las Vegas Metropolitan Police Department Drug Screening/Confirmation Report of Examination distributed on September 20, 2018 for Immunoassay Screen, Amphetamines and Stimulants, and Cannabinoids results.

2.9.4 Amended Reports

When a report is amended, the author of the report will add a statement directly under the report header to state the reason for the amendment and the date of the original report. For example:

- This report is amended to {*reason for the amendment*}. This report supersedes Las Vegas Metropolitan Police Department Blood Alcohol Report of Examination distributed on September 20, 2018.

2.9.5 Reanalysis

On occasion the original analyst on a case is no longer available to testify on their results and the sample must be reanalyzed. The reanalysis report will reference the original report as in the example below. The statement should be listed directly under the report header.

- See Las Vegas Metropolitan Police Department Blood Alcohol Report of Examination by Forensic Scientist Jane Doe, #00000, distributed on September 20, 2018 for original blood alcohol results.

2.10 Report Distribution

Distribution to appropriate parties is handled by the LIMS, the Forensic Lab's support staff, Toxicology Forensic Scientists, Toxicology Supervisor, or Toxicology Manager.

When analysis has been subcontracted or outsourced on cases where some work has been completed in-house, the LVMPD report will be released at the same time as the subcontracted/outsourced report when possible.

For LVMPD cases, a hard copy of the subcontracted/outsourced report will be sent to Records to be scanned into OnBase. An electronic version will be sent to Traffic@LVMPD.com and to the Traffic Investigative Specialist, if applicable. For NHP cases, the subcontracted/outsourced report will be emailed to the main NHP contact (contact information is located at H:\CB\Forensics\Toxicology\Contacts). For other jurisdiction cases, the subcontracted/outsourced report will be sent by US mail by a LEST, or emailed to the requester.

2.11 Toxicology Request Form (LVMPD547)

The Toxicology Request Form for LVMPD blood cases will be scanned into OnBase. The purpose of this is to provide information related to what analysis has been requested by the officer, to assist entities outside of the laboratory (e.g., DA's, Investigative Specialists) with case compilation. The copy of the Toxicology Request Form stored in the case file in LIMS may contain additional information added by the laboratory during the course of analysis.

2.12 Department Drug Testing

The above rules for evidence handling and workflow will apply to Department Samples except as indicated below. All reports will be sent to the Director of Risk Management.

2.12.1 Department Drug Testing samples will be handled as outlined in the LVMPD Department Manual section 8.166 – *Drug Free Workplace*

2.12.2 Reasonable suspicion (RS) and blood samples collected by the Critical Incident Review Team (CIRT) are delivered to the Forensic Laboratory. These samples are logged into the LIMS and are stored in a Toxicology refrigerator.

2.12.3 Department samples that confirm positive for drugs and/or alcohol, and all samples collected in CIRT cases are considered evidence. They are entered into the evidence management system and stored refrigerated until they are transferred to the Evidence Vault.

2.12.4 If the sample confirms positive it will be marked “confidential” in LIMS by the Toxicology Manager or Supervisor.

2.12.5 Disposal
Negative RS samples will not be disposed of until instructed to do so by the Toxicology Manager/designee.

LVMPD FORENSIC LABORATORY
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3.0 Title: ELISA BLOOD SCREENING

Purpose and Scope

This procedure is intended to qualitatively determine the presence of eight analytes/panels of drugs in biological blood samples received into the laboratory, utilizing Enzyme Linked Immunosorbent Assay (ELISA). The panels, their specific analytes, and their cut-off concentrations are:

Drug Class	Cut-off Concentration	Specific Analytes
Amphetamines	20 ng/mL	d-Methamphetamine and MDMA
Benzodiazepines	25 ng/mL	Alprazolam, Diazepam, Nordiazepam, Oxazepam, and Temazepam
Cannabinoids	10 ng/mL	THC Carboxylic Acid
Carisoprodol	500 ng/mL	Carisoprodol and Meprobamate
Cocaine	50 ng/mL	Benzoylecgonine, Cocaethylene
Opiates	10 ng/mL	Codeine, Hydrocodone, and Morphine
Oxycodone	10 ng/mL	Oxycodone and Oxymorphone
PCP	10 ng/mL	Phencyclidine

NOTE: Refer to assay inserts for complete cross-reactivity guide

Principle

Enzyme Linked Immunosorbent Assay (ELISA) Drug Screening is a competitive, solid-phase, heterogeneous immunoassay used for the preliminary identification of drug analytes in blood. Samples, standards, blank, and controls are combined with an enzyme-labeled drug conjugate and added to individual wells coated with a target drug antibody. During the incubation period, free drug and enzyme-labeled conjugate compete for binding sites on the antibody. The wells are washed to remove unbound drug and substrate is added to react with the enzyme-bound drug, producing color. The samples are read with an automatic immunodiagnostic analyzer at a test and reference wavelength. The absorbance is inversely proportional to the amount of drug present in that well.

Instrumentation

The instrument used for the analysis is a Dynex DSX Automated ELISA System. A copy of the instrument parameters is located within the method validation documentation.

Retention of Standards

The current compiled results of a run and its corresponding QC Packet consists of the following:

- Batch Sheet

- Sample Caddy Load List
- Dynex Analysis Results for all Drug Panels
- Instrument Self-Test
- Lot Sheet

Materials

- 16 x 100 mm silanized glass culture tubes*
- 12 x 75 mm glass culture tubes
- 12/13 mm safe-t-flex caps
- Standard control bottles
- Reagent bottles
- DSX reagent tips(white)
- DSX sample tips (blue)

* Other size tubes may be used as necessary

Reagents

Negative Whole Blood (See Section 6.5.1.1 for QC requirements. Store in the freezer. After thawing, store in the refrigerator)

Drug Solutions (See Chapter 3.1 for preparation instructions and Section 6.3.1 for QC requirements. Store in the freezer.)

- Blood Screen Working Solution (Standard and Control):
  1 µg/mL Morphine/Oxymorphone/PCP/THCA, 2.0 µg/mL d-Methamphetamine, 2.5 µg/mL Oxazepam, 5 µg/mL Benzoylecgonine, 50 µg/mL Carisoprodol in methanol

Anti-drug Coated Plates (store per manufacturers' recommendations)

- Benzodiazepine Plate
  OraSure: Benzodiazepines Intercept Micro-Plate EIA Cat. No.: 1110IB or equivalent
- Cannabinoid Plate
  Immunalysis: Cannabinoids (THCA/CTHC) Direct ELISA Kit Cat. No.: 205-0480 or equivalent
- Carisoprodol Plate
  Immunalysis: Carisoprodol Direct ELISA Kit Cat. No.: 231-0480 or equivalent
- Cocaine Plate
  OraSure: Cocaine Metabolite Intercept Micro-Plate EIA Cat. No.: 1122IB or equivalent
- Methamphetamine Plate
  OraSure: Methamphetamines Intercept Micro-Plate EIA Cat. No.: 1104IB or equivalent
- Opiate Plate
  OraSure: Opiates Intercept Micro-Plate EIA Cat. No.: 1150IB or equivalent
- Oxycodone Plate
  Immunalysis: Oxycodone Direct ELISA Kit Cat. No.: 221B-0480 or equivalent
- PCP Plate
  OraSure: PCP Intercept Micro-Plate EIA Cat. No.: 1154IB or equivalent

NOTE: Immediately after opening any Immunalysis assay (Carisoprodol, Oxycodone and THC) identify each plate by coloring the top of the strips. The following coloring reference should be used during casework:

- Carisoprodol – Blue
- Oxycodone – Red
- THC - Green

Kit Reagents (store in the refrigerator)

- Enzyme-labeled Drug Conjugate (OraSure and Immunalysis)
- Substrate Reagent: Tetramethylbenzadine (TMB) (OraSure and Immunalysis)
- OraSure Oral Fluid Negative Calibrator
- OraSure Oral Fluid Cut Off Calibrator
- Immunalysis Synthetic Urine Negative Calibrator
- Immunalysis Synthetic Urine Analyte Specific Positive Control

Kit Reagents (store at room temperature)

- Forensic Specimen Diluent
- Stopping Reagents: Sulfuric Acid (OraSure) and Hydrochloric Acid (Immunalysis)

Remove drug assay kits, reagents, working solutions, and whole blood from the storage location to allow them to equilibrate to room temperature prior to using.

Reagent QC

Methanol working solutions and negative whole blood are QC checked prior to use. All other commercially prepared reagents are verified concurrently with use. All passing criteria for a batch (see **Batch Acceptance Criteria** section) must be met.

Standards, Blank, and Positive Control Preparation

Note: *Silanized vials / test tubes must be used for preparing every standard and control containing THCA prior to the Sample Preparation step below.*

The standards, blank, and positive control must be freshly prepared in blood and diluted with diluent the same day as casework samples on the batch.

Standards, blank, and control are prepared in labeled 16 x 100 mm silanized glass culture tubes using negative whole blood and the specified drug working solution in the volumes listed below.

	Volume of Blood Drug Working Stock Solution	Volume of Negative Whole Blood	Final Volume
Blank	0 µL	1 mL	1 mL
Low Standard	10 µL	1990 µL	2 mL
Cutoff Standard	20 µL	1980 µL	
High Standard	40 µL	1960 µL	
Positive Control	40 µL	1960 µL	

The positive control is prepared in 2 mL aliquots, but may be made in other volumes depending on the amount needed for each run. This positive control will be placed at the beginning, after every ten samples and at the end of the batch.

Sample Preparation

1. Remove blood samples from refrigerator and allow them to equilibrate to room temperature.
2. Ensure the blood vial to be analyzed has the following information placed upon it: Lab number/item number and the analyst's initials.
3. Label glass culture tubes with a LIMS generated barcode label, containing the Lab number/item number and rack position number.
4. Blood vials must be mixed to re-suspend cells prior to dilution.
5. Using the diluter/dispenser, prepare 1:11 dilutions for all samples, standards, blank, and controls, (i.e., aspirate 100 µL of sample and dispense with 1000 µL of forensic specimen diluent) into appropriate labeled glass culture tubes.
6. Flush diluter tip 2-3 times with diluent after each dilution. Wipe diluter tip with a lab wipe.
7. Vortex glass culture tubes on low to homogenize blood and diluents prior to placing on instrument. Ensure that no bubbles are visible in the sample.

Batch Acceptance Criteria

Standards and blank must be run at the beginning of every plate. Before reporting out a result based on the ELISA method, the following criteria must be met:

- The mean optical density (OD) of the blank must be greater than the mean OD of the low standard which must be greater than the mean OD of the cut-off standard which must be greater than the mean OD of the high standard (i.e., blank>low>cut-off>high).
- There must be at least 0.05 separation between the mean OD values of all standards and the blank, without all positive standards resembling blank sample OD values.
- Individual standard/blank OD data readings must not overlap with other standard/blank OD readings.
- The OD values of the cut-off standard must result in a coefficient of variation (CV) of ≤ 20% for mean OD values greater than 0.600 and ≤ 25% for mean OD values less than or equal to 0.600.

If these criteria are not met, the plate is invalid and samples must be reanalyzed for each plate that fails.

- If a positive control fails, the case samples bracketed by the two valid controls immediately before and after the failed control must be reanalyzed. They may be reanalyzed off-line or the entire plate may be reanalyzed. If more than one control fails to give a positive result, the entire batch is invalid and must be reanalyzed for that drug/class.

If batch acceptance criteria are not met on a repeated batch, the analyst will notify the Toxicology Manager or designee. Data from both batches will be reviewed to determine if results can be reported. Technical justification for the use of the data will be noted in the QC packet.

It is noted that even though all acceptance criteria are met within a batch, the drug screen analyst must rely on their training and experience to determine if any anomalies exist that do not fall into the categories discussed above. In these instances the analyst should discuss the anomaly/anomalies with the Toxicology Manager or Supervisor in order to determine if all or part of a batch should be repeated to ensure that the reported results are accurate. If the decision is made to repeat all or part of a batch, the discussion should be documented in the case file.

Note: Data from the Dynex DSX is transferred from the instrument directly into a worksheet in the Forensic Laboratory's LIMS, except for the mean values of the optical density (O.D.) of the cut-off standard and the blank. These values are an average of two results generated for each standard. The DSX software reports these averages, but does not save these values in its text files. Therefore, the LIMS must perform this calculation in order to generate the value for the worksheet. Due to differences in rounding, the value on the worksheet generated by the LIMS can be ±0.001 of the value in the DSX data packet.

Reanalysis

If a plate needs to be reanalyzed it may be reanalyzed on the same day.

Some plates can be analyzed or reanalyzed 24 to 48 hours after the initial sample preparation. See table below for timeline. If analysis on a different day is needed, the sample tubes, standards, blank, and controls must be capped and placed in the refrigerator. The standards, blank, and controls must be analyzed the same day the samples are analyzed.

Plate	Analyze 24 hours after initial preparation	Analyze 48 hours after initial preparation
Benzodiazepine	✓	✓
Cannabinoid	✓	✓
Carisoprodol	NO	NO
Cocaine	✓	✓
Methamphetamine	✓	✓
Opiate	✓	NO
Oxycodone	✓	✓
Phencyclidine	✓	✓

During method development it was observed that an O.D. greater than 3.50 will produce an "OVER" reading. Any casework sample having a value of "OVER" will be repeated. It can be repeated with standards, blank, and controls off-line.

Reporting

The DSX software automatically evaluates the O.D. value of the sample by comparing it to the mean O.D. value of the cutoff. For reporting, samples with O.D. values greater than the mean O.D. value of the cutoff will be reported as negative; samples with O.D. values less than or equal to the mean O.D. value of the cutoff will be reported as positive.

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3.1 Title: DRUG SCREEN – REAGENT PREPARATIONS

Note: Variations to the formulations must be approved by the Forensic Toxicology Manager, or designee.

Blood Drug Working Solutions

Preparation:

1. Standards and controls are prepared from different manufacturers (e.g., Cerilliant (Supelco) used for standards, Cayman Chemical used for controls).
2. THCA stock solutions must be derived from (-)-11-nor-9-Carboxy –Δ9-THC (e.g., Cerilliant (Supelco) item number T-018).
3. Methamphetamine stock solutions must be derived from S(+)-Methamphetamine (e.g., Cerilliant (Supelco) item number M-020).
4. Expiration date is one year from date of preparation or earliest expiration/use by/retest date of a component of the preparation, whichever is sooner.
5. Store in the freezer.

Note: Silanized vials / test tubes must be used for every standard and control containing THCA.

Volume to Pipette	Stock Solution	GC Grade (or better) Methanol Volume	Working Solution Concentration
50 µL	1.0 mg/mL Benzoylecgonine	QS to 10 mL	5 µg/mL
500 µL	1.0 mg/mL Carisoprodol		50 µg/mL
20 µL	1.0 mg/mL S(+)-Methamphetamine		2 µg/mL
10 µL	1.0 mg/mL Morphine		1 µg/mL
25 µL	1.0 mg/mL Oxazepam		2.5 µg/mL
10 µL	1.0 mg/mL Oxymorphone		1 µg/mL
10 µL	1.0 mg/mL Phencyclidine		1 µg/mL
100 µL	100 µg/mL THC-carboxylic acid		1 µg/mL

Quality Control:
See section **6.3.1 Quality Control Checks of Drug Stock and Working Solutions** for Quality Control procedures.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.0 Title: CONFIRMATION TESTING

4.0.1 Purpose and Scope

Confirmation testing is used to determine the identity and concentration of a substance. Currently, the methodology employed for both qualitative identification and quantitative determination is Gas Chromatograph/Mass Spectrometry (GC/MS) selective ion monitoring (SIM) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). GC/MS SIM and LC/MS/MS may also be used to identify and quantify compounds that are not included in the standard screening panel performed at the LVMPD Forensic Laboratory

4.0.2 Sample Preparation

Prior to analysis, allow the standards, controls, negative whole blood, blood evidence, and reagents (except as noted) to equilibrate to room temperature. Ensure the vial of blood to be analyzed has the Lab number/item number placed upon it. The analyst will place their initials upon the vial of the blood to be analyzed.

Vials (extraction, elution, GC/MS, LC/MS/MS) used in the preparation of samples will be labeled consistently and will all bear an identifier traceable to a specific kit in each analyzed batch.

Analysts may assess drug screen data to determine if dilutions are needed on a sample. This assessment is done by comparing O.D. values of the sample to O.D. values of the cutoff calibrator. Dilutions may also be done at the discretion of the analyst.

Casework samples will be pipetted first, followed by methanolic calibration standards and controls, negative matrix, and internal standard. Extractions should take place as soon as possible after pipetting is complete.

The calibration standards, controls, and casework samples shall be extracted on the same day. The same lot of internal standard shall be used.

4.0.3 One-Time Use Solutions

When a solution is prepared to be used in a single examination, its preparation will be recorded with the case documentation instead of being placed in Resource Manager.

4.0.4 Batch Acceptance Criteria

Before reporting out a result based on a confirmatory method, batch acceptance criteria must be met. Batch acceptance criteria may be applied independently for each analyte in a batch. For example, results can be reported for an analyte that

meets batch acceptance criteria even though another analyte on the same batch does not meet batch acceptance criteria. Batch acceptance criteria are outlined below. It is noted that even though all acceptance criteria are met within a batch, the confirmation analyst must rely on their training and experience to determine if any anomalies exist that do not fall into the categories discussed below. In these instances the analyst should discuss the anomaly/anomalies with the Toxicology Manager or Supervisor in order to determine if all or part of a batch should be repeated to ensure that the reported results are accurate. If the decision is made to repeat all or part of a batch, the discussion should be documented in the QC packet, and case file if applicable.

4.0.4.1 Linearity

Each batch shall be calibrated on calibration standards specified in chapters 4.1 and 4.2. The quantitative result of each calibration standard must be at or within ±20% of the target value. One level may be excluded from the calibration. If the level excluded is the lowest calibrator, then any casework with a concentration below that of the next lowest calibrator will be reanalyzed. If the level excluded is the highest calibrator, then any casework with a concentration above that of the next highest calibrator will be reanalyzed. If using ChemStation, the analyst will notate when a calibration standard has been dropped. MassHunter software indicates a dropped calibration standard by an outlined point (rather than a solid point) on the curve graphic. No other notation is needed when using MassHunter software. An r^2 value of greater than or equal to 0.995 must be achieved.

The ratio of target to internal standard for calibration standards responses will be monitored and compared with historical values. If the response ratio is greater than 30% of historical mean values for all calibration standards, the Toxicology Manager or Supervisor will be notified prior to completion of the batch by the analyst.

4.0.4.2 Controls

All methods shall include control samples, if commercially available. Controls shall not be derived from the same lot as the calibration standards. A control shall be run prior to casework samples, at the end of each batch, and after every 10 samples throughout the batch. The controls will be of varying concentrations within the range of the curve. At a minimum, positive controls will be run at concentrations equal to the lowest calibration standard and equal to the highest calibration standard on each batch. Positive controls shall be no greater than ±20% of the target value.

Each control shall be evaluated independently and the failure of a control for a single analyte does not invalidate the control of other analytes within that assay. If a positive control fails, the case samples bracketed by the two valid controls immediately before and after the failed control shall be repeated. Moreover, in order to report a positive result, the positive result shall be at a concentration that is equal to or within the range of positive controls that meet QC requirements within the batch. If the low positive control does not meet

quantitative criteria, specimens with an indication of analyte (i.e., chromatographic peaks with a quantitative value below the reporting threshold) shall be repeated to confirm negative results. If more than one positive control fails for a single analyte, all samples must be repeated. In the event of control failure(s), case samples with no indication that the analyte is present (i.e., no quantitation ion peak) do not need to be repeated and may be reported as "none detected".

4.0.4.3 Negative Control

A negative control consisting of a drug free matrix is spiked with internal standard and run after the highest calibration standard. The negative control also serves as a carryover check for each batch. The negative control shall produce a negative result. A result is defined as negative when the abundance/area counts of the target ion is less than 10% relative to that of the target ion of the lowest calibration standard. If the negative control has abundance/area counts equal to or greater than 10% of the lowest calibration standard, but does not meet retention time criteria listed in section 4.0.5.1, the result will be deemed negative.

4.0.5 Qualitative Identification of Analytes

4.0.5.1 Retention time

The retention time of analytes should be no greater than ±2 % of the retention time as established by calibration samples and controls. If an analyte in a casework sample falls outside of this range due to overloading, then the sample will be repeated after the sample has been diluted and re-extracted. Relative Retention Time (retention time of the analyte target ion divided by the retention time of the internal standard target ion) should remain consistent throughout each batch.

4.0.5.2 Qualifying ion correlation

For electron impact (EI) analysis, each analyte must have a primary ion and two qualifying ions. Internal Standards must have a primary ion and one qualifying ion.

For chemical ionization (CI) analysis each analyte must have a primary ion and at least one qualifying ion. Internal Standards shall have a primary ion and one qualifying ion.

For MS/MS analysis, each analyte must have a primary transition and at least one qualifying transition. Internal Standards must have a primary transition (a qualifying transition is not required).

4.0.5.3 Ion/transition ratios

Qualifying ion/transition ratios generally should be no greater than ±20 % (for EI and MS/MS methods) of the ion ratios of the corresponding control or calibrators. However, it is recognized that some ion/transition ratios are concentration dependent and that comparison to a calibrator or control of similar concentration may be necessary, rather than comparison with a value

calculated from a single known sample or an average calculated from all calibration samples over the entire quantitative range.

4.0.6 Manual Integration

It is recognized that peak integration which is performed automatically by the instrument software may not be satisfactory due to interferences that are a routine part of biological sample analyses. In such cases, manual integration may be used.

MassHunter software will place an asterisk next to peaks that have been manually integrated and display the peaks in a different color. No further notation is needed by the analyst when using MassHunter.

Peaks integrated manually will be reviewed during the technical review. A completed technical review will indicate agreement with the execution of the manual integration.

4.0.7 Re-injections

There may be situations in which samples may need to be re-injected (e.g., poor chromatography, interference, failed ion ratios). Samples may be re-injected the following day if no major changes to the system have been made (e.g., source cleaning, autotune, etc.). Document the re-injection on the chromatogram with the reason for the re-injection. The sample name of the re-injected vial is the same as the original injection data file except that it is appended with “R” for “re-injection.”

Because the negative control specimen also serves as a carryover check, re-injecting the negative control to remove carryover is not acceptable.

4.0.7.1 Re-injections Following Specimens with High Concentrations

Agilent ChemStation software utilizes Intelligent Sequencing which automatically injects a blank after a specimen with a concentration above the level specified in the method. There are circumstances when this feature does not function appropriately (e.g., high concentration of analyte overloads the column and no quantitative result is calculated). In such circumstances, samples analyzed directly after those with concentrations exceeding the carryover check level must be re-injected to confirm positive results, if the result is being reported.

When analyzing samples on the LC/MS/MS with MassHunter software, casework and Department samples analyzed after those with concentrations exceeding the highest standard by more than 20% must be re-injected to confirm positive results, if the result is being reported.

4.0.8 Reporting

A report that is issued represents a summary of the analytical findings, identifies the substance(s) tested, and lists the amount or “none detected” if no substance in a drug class is detected at or above the cut-off. Quantitative results for all analytes where the lowest calibration standard is less than 1.0 ng/mL (e.g., 6-AM, buprenorphine, norbuprenorphine, fentanyl) are truncated to two decimal places. Quantitative results for all other analytes are truncated to one decimal place.

Measurement uncertainty is reported for all positive quantitative results. Standard rules of rounding are used to calculate measurement uncertainty results.

Quantitative results for drug analytes must not be reported below the cutoff concentration. For analytes with linear calibration models, results may be reported at a concentration of up to 20% greater than the highest standard if the method has been validated to be linear at that level. For analytes with non-linear calibration models (e.g., quadratic), results may be reported at concentrations up to and including the highest calibration standard.

If the results from a sample exceed the highest calibrated level by more than twenty percent, then the analyst will follow the guidelines listed below:

- If a dilution was performed, divide the result by the dilution factor. If that result is within the calibration range, report the result listed on the chromatogram.
- If any blood sample has sufficient quantities for multiple analyses, the sample will be repeated after the sample has been diluted and re-extracted.
- If any blood sample has insufficient quantity to perform a dilution, then the results will be reported out as greater than the highest calibration standard.

Laboratory management has the discretion to allow changes in reporting guidelines on a case by case basis. The approval for the change to the reporting guideline must be documented in the case record.

4.0.9 Measurement Uncertainty

Measurement uncertainty documents are located in Qualtrax at Documents\LVMPD\Forensic Lab\Toxicology\Measurement Uncertainty. The measurement uncertainty will be reviewed and/or recalculated every two years and will be recalculated if there are procedural changes to the method that affect the quantitative measurement. The measurement uncertainty may be reviewed and/or recalculated at any time at the discretion of the Toxicology Manager.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.1.01 Title: CONFIRMATION – AMPHETAMINES AND STIMULANTS IN BLOOD

Purpose and Scope: This procedure is used to quantitatively determine amphetamine, methylenedioxyamphetamine, methamphetamine, methylenedioxymethamphetamine, phentermine, and methylphenidate in whole blood.

Principle: The deuterium labeled analog of each analyte is added to each sample as an internal standard. The analytes and internal standards are extracted from whole blood using a liquid-liquid extraction technique and analyzed by LC/MSMS.

Materials:

- 16 x 100 mm glass screw-top tubes and caps*
- Disposable glass Pasteur pipettes
- LC/MS/MS autosampler vials with inserts and caps

*Other size tubes may be used as necessary.

Reagents:

Chemicals:

- Sodium phosphate, tribasic, ACS grade or higher
- Distilled/purified water
- 1-Chlorobutane, LC grade or higher
- Hydrochloric acid, ACS grade or higher
- 2-Propanol, LC grade or higher
- Formic acid, LCMS grade
- Water, LCMS grade
- Methanol, LC grade or higher
- Acetonitrile, LCMS grade

Reagent solutions (see Chapter 4.3 for preparation, QC, and storage instructions):

- 0.2 M sodium phosphate, tribasic
- 0.2% (v/v) Hydrochloric acid in 2-propanol
- 0.1% (v/v) Formic acid in water
- 0.1% (v/v) Formic acid in acetonitrile

Drug solutions (see Chapter 4.2 for preparation, QC, and storage instructions):

- Calibration standard working solution level 1 – 1 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH
- Calibration standard working solution level 2 – 10 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH
- Control working solution level 1 – 1 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH

- Control working solution level 2 – 10 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH
- Internal standard working solution – 1 µg/mL AMP-D_{11}, MDA-D_5, METH-D_{11}, MDMA-D_5, PHEN-D_5, and MPH-D_9

Calibrators and Controls:

Calibrators are prepared in 1.0 mL aliquots at each of the concentrations listed below in labeled 16 x 100 mm glass screw-top tubes using negative whole blood and the specified calibration standard working solutions.

Controls are prepared in the same concentrations as calibrators. A control is run after the negative control, after every 10 samples, and at the end of the batch.

Calibrator / Control	Final Concentration of AMP/MDA/METH/MDMA/ PHEN/MPH (ng/mL)	Volume of Working Solution Level 1 (1 µg/mL)	Volume of Whole Blood
1	20	20 µL	1000 µL
2	50	50 µL	1000 µL
3	75	75 µL	1000 µL
4	100	100 µL	1000 µL
Calibrator / Control	**Final Concentration of AMP/MDA/METH/MDMA/ PHEN/MPH (ng/mL)**	**Volume of Working Solution Level 2 (10 µg/mL)**	**Volume of Whole Blood**
5	250	25 µL	1000 µL
6	500	50 µL	1000 µL
7	750	75 µL	1000 µL
8	1000	100 µL	1000 µL
Negative Control	0	0	1000 µL

Preparation:

1. Pipet 1.0 mL of each casework blood specimen into a labeled 16 x 100 mm glass screw-top tube.
2. Prepare calibrators and controls as described above.
3. Add 100 µL of internal standard working solution to each tube.
4. Add 2 mL of 0.2 M sodium phosphate, tribasic and vortex.
5. Add 6 mL of 1-chlorobutane to each tube and vortex.
6. Cap and rotate tubes for at least 20 minutes.
7. Centrifuge at ~3000 rpm for at least 30 minutes.
8. Transfer the upper organic layer to appropriately labeled tubes.
9. Add 100 µL of 0.2% (v/v) HCl in 2-propanol to each tube and vortex.

10. Evaporate samples under nitrogen to dryness at ~40˚ C. **Do not over dry.**

Reconstitution:

11. Add 1.5 mL of 0.1% (v/v) formic acid in water and vortex.
12. Transfer the contents of each tube into an autosampler vial. Cap and transfer to the autosampler tray for LC/MSMS analysis.

LC/MS/MS Analysis:

LVMPD Instrument	Tox #1 LCMSMS
Instrument Make/Model	Agilent 6420 Triple Quadrupole LC/MS
Software	Agilent MassHunter
Acquisition Method	AMPSTIM_B.m
Data Analysis Method	AMPSTIM_B.m
Reporting Method	AMPSTIM_B.m

LC Parameters:

Multisampler Temperature	4.0 °C - Room Temperature
Injection Volume	1 - 5 µL (e.g., 2 µL)
Column	Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 50 mm, 2.7 µm)
Column Temperature	50 °C
Needle Wash	10 s
Needle Wash Solution	75:25 Methanol:Water
Mobile Phase A	0.1% (v/v) Formic Acid in Water
Mobile Phase B	0.1% (v/v) Formic Acid in Acetonitrile
Flow Rate	0.5 mL/min
Gradient:	

Time (Minutes)	**% Aqueous** 0.1% formic acid in water	**% Organic** 0.1% formic acid in acetonitrile
Initial	98	2
2	95	5
4	90	10
6	40	60
6.5	10	90
8	10	90
8.5 (Stop)	98	2

Post Time 2.5 minutes

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

MSD Parameters:

Parameter	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	350 °C
Gas Flow	13.0 L/min
Nebulizer Pressure	30 psi
Capillary	1500 V

Analyte	Quantitation Transition	Qualifier Transition
AMP-D11	147.2 → 98.1	n/a
AMP	136.1 → 91.1	136.1 → 119.0
MDA-D5	185.1 → 168.1	n/a
MDA	180.1 → 163.0	180.1 → 105.1
METH-D11	161.2 → 97.1	n/a
METH	150.1 → 91.1	150.1 → 119.0
MDMA-D5	199.1 → 165.0	n/a
MDMA	194.1 → 163.0	194.1 → 105.1
PHEN-D5	155.2 → 96.1	n/a
PHEN	150.1 → 91.1	150.1 → 133.1
MPH-D9	243.2 → 93.2	n/a
MPH	234.1 → 84.2	234.1 → 56.2

Calibration Models:

Analyte	Model Type	Origin	Weighting
Amphetamine	Quadratic	Ignore	1/x
MDA	Quadratic	Ignore	1/x
Methamphetamine	Quadratic	Ignore	1/x
MDMA	Linear	Ignore	1/x
Phentermine	Quadratic	Ignore	1/x
Methylphenidate	Quadratic	Ignore	1/x

Note: The LVMPD Forensic Laboratory does not distinguish enantiomers of AMP, MDA, METH, MDMA, or MPH when reporting drug confirmation results.

Processed Sample Stability:

Room Temperature – 72 hours
Refrigerator – 72 hours

LVMPD FORENSIC LABORATORY TECHNICAL PROCEDURES TOXICOLOGY

4.1.02 Title: CONFIRMATION – COCAINE / COCAETHYLENE / BENZOYLECGONINE IN BLOOD

Purpose and Scope: This procedure is used to quantitatively determine cocaine, cocaethylene, and benzoylecgonine in whole blood.

Principle: The deuterium labeled analog of each analyte is added to each sample as an internal standard. Protein precipitation with acetonitrile is used to prepare the whole blood specimens for analysis. An LC/MSMS is used for the identification and quantitation of analytes.

Materials:

- 16 x 125 mm glass tubes*
- 16 x 100 mm glass tubes*
- LC/MSMS autosampler vials with caps

*Other size tubes may be used as necessary.

Reagents:

Chemicals:

- Acetonitrile, HPLC grade or better
- Hydrochloric acid
- Methanol
- 0.1% Formic Acid in acetonitrile, LCMS grade
- 0.1% Formic acid in water, LCMS grade

Reagent solutions (unless specified below, see Chapter 4.3 for preparation, QC, and storage instructions):

- 1% Hydrochloric acid in methanol
  - Prepare fresh for one-time use
  - QC: Concurrently with use
- 5% (v/v) Acetonitrile with 0.1% formic acid in water with 0.1% formic acid
  - Prepare fresh for one-time use
  - QC: Concurrently with use

Drug solutions (see Chapter 4.2 for preparation, QC, and storage instructions):

- Calibration standard working solution level 1 – 1 µg/mL COC/CE and 5 µg/mL BZE
- Calibration standard working solution level 2 – 10 µg/mL COC/CE and 50 µg/mL BZE
- Control working solution level 1 – 1 µg/mL COC/CE and 5 µg/mL BZE
- Control working solution level 2 – 10 µg/mL COC/CE and 50 µg/mL BZE
- Internal standard working solution – 2 µg/mL COC-D_3/CE-D_3 and 10 µg/mL BZE-D_3

Calibrators and Controls:

Calibrators are prepared in 1.0 mL aliquots at each of the concentrations listed below in labeled 16 x 125 mm glass tubes using negative whole blood and the specified calibration standard working solutions.

Controls are prepared in the same concentrations as calibrators. A control is run after the negative control, after every 10 samples, and at the end of the batch.

Calibrator / Control	Final Concentration of COC, CE / BZE (ng/mL)	Volume of COC, CE / BZE Working Solution Level 1 (1 / 5 µg/mL)	Volume of Whole Blood
1	10 / 50	10 µL	1000 µL
2	50 / 250	50 µL	1000 µL
3	100 / 500	100 µL	1000 µL
Calibrator / Control	**Final Concentration of COC, CE / BZE (ng/mL)**	**Volume of COC, CE / BZE Working Solution Level 2 (10 / 50 µg/mL)**	**Volume of Whole Blood**
4	250 / 1250	25 µL	1000 µL
5	500 / 2500	50 µL	1000 µL
6	1000 / 5000	100 µL	1000 µL
Negative Control	0	0	1000 µL

Preparation:

1. Pipet 1.0 mL of each casework blood specimen into a labeled 16 x 125 mm glass tube.
2. Prepare calibrators and controls as described above.
3. Add 50 µL of internal standard working solution to each tube and vortex.

Protein Precipitation:

4. Add 2 mL of cold acetonitrile (i.e., stored in freezer) while vortexing.
5. Centrifuge at ~3000 rpm for at least 10 minutes.
6. Transfer supernatant to appropriately labeled 16 x 100 mm glass culture tubes.
7. Add 100 µL of 1% hydrochloric acid in methanol and vortex.
8. Transfer tubes to an evaporator bath and evaporate to dryness at ~50°C under a gentle stream of nitrogen.

Reconstitution:

9. Add 1 mL of 5% (v/v) acetonitrile with 0.1% formic acid in water with 0.1% formic acid and vortex.
10. Transfer the contents of each tube into an autosampler vial. Cap and transfer to the autosampler tray for LC/MS/MS analysis.

LC/MS/MS Analysis:

LVMPD Instrument	Tox #1 LCMSMS
Instrument Make/Model	Agilent 1260 Infinity LC and 6420 Triple Quadrupole LC/MS
Software	Agilent MassHunter
Acquisition Method	COC_B.m
Data Analysis Method	COC_B.m
Reporting Method	COC_B.m

LC Parameters:

Multisampler Temperature	4.0 °C - Room Temperature
Injection Volume	1 - 5 µL (e.g., 2 µL)
Column	Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 50 mm, 2.7 µm)
Column Temperature	30 °C
Needle Wash	10 s
Needle Wash Solution	75:25 Methanol:Water
Mobile Phase A	0.1% (v/v) Formic Acid in Water
Mobile Phase B	0.1% (v/v) Formic Acid in Acetonitrile
Flow Rate	0.5 mL/min

Gradient:

Time (Minutes)	**% Aqueous** 0.1% formic acid in water	**% Organic** 0.1% formic acid in acetonitrile
Initial	95	5
5	60	40
5.5	10	90
7	10	90
7.5 (Stop)	95	5

Post Time 3 minutes

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

MSD Parameters

Parameter	**Value**
Ionization	ESI
Polarity	Positive
Gas Temperature	350 °C
Gas Flow	12 L/min
Nebulizer Pressure	15 psi
Capillary	1500 V

Analyte	Quantitation Transition	Qualifier Transition
BZE-D3	293.2 → 171.1	n/a
BZE	290.1 → 168.0	290.1 → 105.0
COC-D3	307.2 → 185.1	n/a
COC	304.2 → 182.1	304.2 → 82.1
CE-D3	321.1 → 199.1	n/a
CE	318.2 → 196.1	318.2 → 82.1

Calibration Models:

Analyte	Model Type	Origin	Weighting
Benzoylecgonine	Linear	Ignore	1/x
Cocaine	Linear	Ignore	1/x
Cocaethylene	Linear	Ignore	1/x

Processed Sample Stability:

Room Temperature – 72 hours
Refrigerator – 72 hours

LVMPD FORENSIC LABORATORY TECHNICAL PROCEDURES TOXICOLOGY

4.1.03 Title: CONFIRMATION – CANNABINOIDS IN BLOOD

Purpose and Scope: This procedure is used to quantitatively determine the psychoactive component of marijuana (Δ9-tetrahydrocannabinol), an active metabolite 11-hydroxy-Δ9-tetrahydrocannabinol, and the major inactive metabolite tetrahydrocannabinol-carboxylic acid in whole blood.

Principle: The deuterium labeled analog of each analyte is added to each sample as an internal standard. The compounds and internal standards are extracted from whole blood using a liquid-liquid extraction technique and analyzed by LC/MS/MS.

Materials:

- 16 x 100 mm silanized glass screw-top tubes and caps*
- 16 x 100 mm silanized glass culture tubes*
- Disposable glass Pasteur pipettes
- LC-MS/MS autosampler vials with inserts and caps

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Water, LC-MS grade
- Acetic acid, glacial
- Hexane
- Ethyl acetate

Acetonitrile, LC-MS grade

Reagent Solutions

Prepare fresh daily:

- 10% Acetic acid solution – 9:1 water:glacial acetic acid
  QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Organic extraction solvent – 9:1 hexane:ethyl acetate
  QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.

Drug solutions (see Chapter 4.2 for preparation and storage instructions. See Section 6.3.1 for QC instructions):

- Calibration standard working solution level 1 – 0.1 µg/mL THC, 11-OH-THC / 0.5 µg/mL THCA in methanol.
- Calibration standard working solution level 2 – 1 µg/mL THC, 11-OH-THC / 5 µg/mL THCA in methanol.

- Control working solution level 1 – 0.1 µg/mL THC, 11-OH-THC / 0.5 µg/mL THCA in methanol.
- Control working solution level 2 – 1 µg/mL THC, 11-OH-THC / 5 µg/mL THCA in methanol.
- Internal standard working solution – 0.1 µg/mL THC-D_3, 11-OH-THC-D_3 / 0.5 µg/mL THCA-D_3 in methanol.

Calibrators and Controls:

Calibrators are prepared in 1.0 mL aliquots at each of the concentrations listed below in labeled 16 x 100 mm silanized glass screw-top tubes using negative whole blood and the specified calibration standard working solution.

Controls are prepared in the same concentrations as calibrators. A control is run after the negative control, after every 10 samples, and at the end of the batch.

Calibrator / Control	Final Cannabinoid Concentration (ng/mL) THC, 11-OH-THC / THCA	Volume of Cannabinoid Calibration Standard Working Solution Level 1 (0.1 µg/mL THC, 11-OH-THC / 0.5 µg/mL THCA)	Volume of Whole Blood
1	1 / 5	10 µL	1000 µL
2	5 / 25	50 µL	1000 µL
3	10 / 50	100 µL	1000 µL
Calibrator / Control	**Final Cannabinoid Concentration (ng/mL) THC, 11-OH-THC / THCA**	**Volume of Cannabinoid Calibration Standard Working Solution Level 2 (1 µg/mL THC, 11-OH-THC / 5 µg/mL THCA)**	**Volume of Whole Blood**
4	25 / 125	25 µL	1000 µL
5	50 / 250	50 µL	1000 µL
6	100 / 500	100 µL	1000 µL
Negative Control	0	0	1000 µL

Preparation:

1. Pipet 1.0 mL of each casework blood specimen into a labeled 16 x 100 mm silanized glass screw-top tube.
2. Prepare calibrators and controls as described above.
3. Add 100 µL of internal standard working solution to each tube.
4. Add 2 mL of water to each tube and vortex.
5. Add 800 µL of 10% acetic acid and vortex.
6. Add 6 mL of 9:1 hexane:ethyl acetate solution, cap, vortex, and rock/rotate tubes for 20 minutes.

7. Centrifuge at ~3000 rpm for at least 30 minutes.
8. Transfer the upper organic layer to appropriately labeled silanized tubes.
9. Transfer the tubes to an evaporator bath and evaporate to dryness at 30˚C under a gentle stream of nitrogen.

Reconstitution:

10. Add 50 µL of acetonitrile, LC-MS grade to each tube and vortex.
11. Add 50 µL of water, LC-MS grade to each tube and vortex.
12. Transfer the contents of each tube into an autosampler vial equipped with an insert. Cap and transfer to the autosampler tray for LC/MS/MS analysis.

LC/MS/MS Analysis:

LVMPD Instrument	Tox #1 LC/MS/MS
Instrument Make/Model	Agilent 6420 LC/MS/MS
Software	Agilent MassHunter
Acquisition Method	Cannabinoids_B.m
Data Analysis Method	Cannabinoids_B.m
Reporting Method	Cannabinoids_B.m

LC Parameters:

Multisampler Temperature	4.0 °C - Room Temperature
Injection Volume	2.0 - 30.0 µL (e.g., 10.0 µL)
Column	Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 50 mm, 2.7 µm)
Column Temperature	40 °C
Needle Wash	10 s
Needle Wash Solution	1:1:1:1 Methanol:Water:Acetonitrile:2-Propanol
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Flow Rate	0.5 mL/min

Gradient:

Time (Minutes)	**% Aqueous** 0.1% formic acid in water	**% Organic** 0.1% formic acid in acetonitrile
Initial	60	40
1.0	60	40
7.0	5	95
10.0	5	95
10.5 (Stop)	60	40

Post Time 3.0 minutes

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

MSD Parameters

Parameter	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	320 °C
Gas Flow	11 L/min
Nebulizer Pressure	30 psi
Capillary	5,500 V

Analyte	Quantitation Transition	Qualifier Transition
11-OH-THC-D_3	334.2 → 196.1	n/a
11-OH-THC	331.2 → 193.1	331.2 → 201.0
THCA-D_3	348.2 → 302.1	n/a
THCA	345.2 → 299.1	345.2 → 193.1
THC-D_3	318.2 → 196.1	n/a
THC	315.2 → 193.0	315.2 → 123.0

Calibration Models:

Analyte	Model Type	Origin	Weighting
11-OH-THC	Linear	Ignore	1/x
THCA	Linear	Ignore	1/x
THC	Linear	Ignore	1/x

Processed Sample Stability:

Room Temperature – 72 hours
Refrigerator – 72 hours

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TECHNICAL PROCEDURES
TOXICOLOGY

4.1.04 Title: CONFIRMATION – NARCOTIC ANALGESICS IN BLOOD

Purpose and Scope: This procedure is used to quantitatively determine the concentration of 6-Acetylmorphine, Buprenorphine, Codeine, Fentanyl, Hydrocodone, Hydromorphone, Methadone, Morphine, Norbuprenorphine, O-Desmethyltramadol, Oxycodone, Oxymorphone, and Tramadol and qualitatively determine the presence of Mitragynine and its metabolites/stereoisomers in whole blood.

Principle: The deuterium labeled analog of each analyte is added to each sample as an internal standard. The analytes and internal standards are extracted from whole blood and analyzed by LC/MSMS.

Materials:

- SPE columns (Agilent Bond Elut Plexa PCX #12108206, or equivalent)
- 16 x 100 mm silanized glass culture tubes*
- Autosampler vials, caps and silanized/deactivated inserts.
  *other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled/purified water
- Water, LC grade or higher
- Methanol, LC grade or higher
- Ethyl Acetate, HPLC grade or higher
- Isopropanol, HPLC grade or higher
- Ammonium hydroxide, ACS grade or higher
- 2% Formic Acid, LC grade or higher
- Formic Acid (LC grade)

Reagent Solutions: (Unless specified below, see Chapter 4.3 for preparation, QC, and storage instructions):

- Phosphate buffer, 100 mM, pH 6.0
- Water with 0.1% formic acid
- Methanol with 0.1% formic acid
- (80:10:10) Acetonitrile:Isopropanol:Methanol

Prepare Fresh Daily: QC Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.

- 2% Formic Acid Solution—98:2 water:formic acid (May use commercially prepared reagent in lieu of)
- 2% Formic Acid solution in Methanol—70:30 methanol:2% formic acid

- Eluting Solution – ethyl acetate:isopropanol:ammonium hydroxide (80:20:5). Add NH_4OH shortly before elution.
- Reconstitution Solution – water:methanol (90:10)

Drug Solutions (see Chapter 4.2 for preparation and storage instructions. See Section 6.3.1 for QC instructions):

- Calibration standard working solution Level 1– 0.01 µg/mL fentanyl; 0.05 µg/mL 6-acetylmorphine, buprenorphine, norbuprenorphine; 0.1 µg/mL oxymorphone, hydromorphone; 0.5 µg/mL morphine, codeine, oxycodone, hydrocodone, o-desmethyltramadol, tramadol, mitragynine; 1 µg/mL methadone in methanol.
- Calibration standard working solution Level 2– 0.1 µg/mL fentanyl; 0.5 µg/mL 6-acetylmorphine, buprenorphine, norbuprenorphine; 1 µg/mL oxymorphone, hydromorphone; 5 µg/mL morphine, codeine, oxycodone, hydrocodone, o-desmethyltramadol, tramadol, mitragynine; 10 µg/mL methadone in methanol.
- Control working solution Level 1– 0.01 µg/mL fentanyl; 0.05 µg/mL 6-acetylmorphine, buprenorphine, norbuprenorphine; 0.1 µg/mL oxymorphone, hydromorphone; 0.5 µg/mL morphine, codeine, oxycodone, hydrocodone, o-desmethyltramadol, tramadol, mitragynine; 1 µg/mL methadone in methanol.
- Control working solution Level 2– 0.1 µg/mL fentanyl; 0.5 µg/mL 6-acetylmorphine, buprenorphine, norbuprenorphine; 1 µg/mL oxymorphone, hydromorphone; 5 µg/mL morphine, codeine, oxycodone, hydrocodone, o-desmethyltramadol, tramadol, mitragynine; 10 µg/mL methadone in methanol.
- Internal standard working solution – 0.02 µg/mL fentanyl-D_5; 0.1 µg/mL 6-acetylmorphine-D_6, buprenorphine-D_4, norbuprenorphine-D_3; 0.2 µg/mL oxymorphone-D_3, hydromorphone-D_6; 1 µg/mL morphine-D_6, codeine-D_6, oxycodone-D_6, hydrocodone-D_6, , tramadol-^{13}C-D_3, o-desmethyltramadol-D_6, mitragynine-D_3 and methadone-D_3 in methanol.

Calibrators and Controls:

Calibrators are prepared in 1 mL aliquots at each of the concentrations listed below in labeled 16 x 100 mm silanized glass culture tubes using negative whole blood and the specified calibration standard working solution.

Controls are prepared in the same concentrations as calibrators. A control is run prior to case samples, after every 10 samples throughout the batch, and at the end of the run.

Calibrator / Control	Final Conc. (ng/mL) of Narcotic Analgesic Working Solution Level 1 (0.01 / 0.05 / 0.1 / 0.5 / 1 µg/mL) FEN / 6-AM, BUP, NBUP / OXM, HYM / MOR, COD, OXC, ODT, HYC, TRM, MG / MTD	Volume Narcotic Analgesics Working Solution Level 1 (0.01 / 0.05 / 0.1 / 0.5 / 1 µg/mL) FEN / 6-AM, BUP, NBUP / OXM, HYM / MOR, COD, OXC, ODT, HYC, TRM, MG / MTD	Volume of Whole Blood
1	0.1 / 0.5 / 1 / 5 /10	10 µL	1000 µL
2	0.5 / 2.5 / 5 / 25 / 50	50 µL	1000 µL
3	1 / 5 / 10 / 50 / 100	100 µL	1000 µL
4	1.5 / 7.5 / 15 / 75 / 150	150 µL	1000 µL
Calibrator / Control	**Final Conc. (ng/mL) of Narcotic Analgesic Working Solution Level 2 (0.1 / 0.5 / 1 / 5 / 10 µg/mL) FEN / 6-AM, BUP, NBUP / OXM, HYM / MOR, COD, OXC, ODT, HYC, TRM, MG / MTD**	**Volume Narcotic Analgesics Working Solution Level 2 (0.1 / 0.5 / 1 / 5 / 10 µg/mL) FEN / 6-AM, BUP, NBUP / OXM, HYM / MOR, COD, OXC, ODT, HYC, TRM, MG / MTD**	**Volume of Whole Blood**
5	3 / 15 / 30 / 150 / 300	30 µL	1000 µL
6	5 / 25 / 50 / 250 / 500	50 µL	1000 µL
7	7 / 35 / 70 / 350 / 700	70 µL	1000 µL
8	8 / 40 / 80 / 400 / 800	80 µL	1000 µL
Negative Control	0	0	1000 µL

Preparation:

1. Pipet 1 mL of each casework blood specimen into a labeled 16 x 100 mm silanized glass culture tube.
2. Prepare calibrators and controls as described above.
3. Add 50 µL of internal standard working solution to each tube.
4. Add 4 mL of 100 mM phosphate buffer 6.0 and vortex. Centrifuge for at least 10 minutes at ~3000 rpm.
5. Place SPE extraction columns into an extraction manifold.
6. Condition Bond Elut Plexa PCX cartridge with 0.5 mL methanol, soak, let drip. Once dripping stops, apply low pressure to force out remaining methanol.
7. Load samples and run through SPE columns.
8. Wash SPE columns with:
    a. 2 mL of 2% formic acid
    b. An additional 2 mL of 2% formic acid
    c. 3 mL of 70 MeOH:30 of 2% formic acid
9. Dry SPE columns for 5-10 minutes under high pressure.
10. Place labeled 16x100 mm silanized glass culture tubes in manifold under each SPE column and elute with:

a. 0.75 mL of eluting solution (ethyl acetate:isopropanol:ammonium hydroxide (80:20:5))
b. An additional 0.75 mL of eluting solution
c. Allow eluate to soak the sorbent bed with each aliquot. Let the eluate drip into the collection vials under gravity. When the dripping stops, apply low pressure to extract eluate from the smallest pores.

Reconstitution:

11. Evaporate to dryness under a stream of nitrogen at a maximum of 45 °C.
12. Add 200 µL of reconstitution solution (water:methanol (90:10)) to each vial; vortex, and transfer into autosampler vials with silanized vial inserts.
    a. Solvents must be LC/MS grade or better
    b. Samples may be centrifuged prior to transfer into autosampler vials, if desired.

LC/MS/MS Analysis:

LVMPD Instrument:	TOX #2 LC/MSMS
Instrument Make/Model:	Agilent 1260 LC, 6420 MS/MS
Software:	Agilent MassHunter
Acquisition Method:	Narcotic-Analgesics_B.m
Data Analysis Method:	Narcotic-Analgesics_B.m
Reporting Method:	Narcotic-Analgesics_B.m

LC Parameters:

Multisampler Temperature:	4.0 °C - Room Temperature
Injection Volume:	2.0 - 30.0 µL (e.g., 10.0 µL)
Column:	Agilent Poroshell 120 Phenyl Hexyl (2.1 x 50 mm, 2.7 µm)
Column Temperature:	45 °C
Needle Wash:	25 s
Needle Wash Solution:	80:10:10 – Acetonitrile:Isopropanol:Methanol
Mobile Phase A:	Water with 0.1% Formic Acid
Mobile Phase B:	Methanol with 0.1 % Formic Acid
Flow Rate	0.75 mL/min

Gradient

Time (Minutes)	**% Aqueous** Water with 0.1% formic acid	**% Organic** Methanol with 0.1% formic acid
Initial	93	7
2.2	20	80
3.5	5	95
4.0	85	15
5.0 Stop	85	15
Post Time	2.0 minutes	

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

MSD Parameters

Time Segment #2 Parameters	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	260 °C
Gas Flow	12 L/min
Nebulizer Pressure	15 psi
Capillary	1,000 V

Time Segment #3 Parameters	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	260 °C
Gas Flow	12 L/min
Nebulizer Pressure	25 psi
Capillary	1,300 V

Time Segment #4 Parameters	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	260 °C
Gas Flow	10 L/min
Nebulizer Pressure	20 psi
Capillary	1,000 V

Time Segment #5 Parameters	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	260 °C
Gas Flow	7 L/min
Nebulizer Pressure	25 psi
Capillary	1,000 V

Analyte	Quantitation Transition	Qualifier Transition
Morphine-D6	292.2 → 152.1	n/a
Morphine	286.2 → 152.1	286.2 → 165.1
Oxymorphone-D3	305.2 → 230.1	n/a
Oxymorphone	302.1 → 227.1	302.1 → 198.1
Hydromorphone-D6	292.2 → 185.1	n/a
Hydromorphone	286.2 → 185.1	286.2 → 157.1
Codeine-D6	306.2 → 152.1	n/a
Codeine	300.2 → 152.1	300.2 → 165.1
O-Desmethyltramadol-D6	256.2 → 64.2	n/a
O-Desmethyltramadol	250.2 → 58.2	250.2 → 42.2
Oxycodone-D6	322.2 → 262.2	n/a
Oxycodone	316.2 → 241.1	316.2 → 256.2
6-Acetylmorphine-D6	334.2 → 165.1	n/a
6-Acetylmorphine	328.2 → 165.1	328.2 → 211.1
Hydrocodone-D6	306.2 → 202.1	n/a
Hydrocodone	300.2 → 199.1	300.2 → 128.1
Tramadol-^{13}C-D3	268.2 → 58.2	n/a
Tramadol	264.2 → 58.2	264.2 → 42.2
Norbuprenorphine-D3	417.3 → 83.2	n/a
Norbuprenorphine	414.3 → 83.2	414.3 → 55.2
Fentanyl-D5	342.3 → 188.2	n/a
Fentanyl	337.2 → 188.2	337.2 → 105.1
Buprenorphine-D4	472.3 → 59.2	n/a
Buprenorphine	468.3 → 55.2	468.3 → 396.2
Mitragynine-D3	402.2 → 177.1	n/a
Mitragynine	399.2 → 174.1	399.2 → 226.2
Methadone-D3	313.2 → 268.2	n/a
Methadone	310.2 → 265.2	310.2 → 105.1

Calibration Models:

Analyte	Model Type	Origin	Weighting
Morphine	Quadratic	Ignore	1/x
Oxymorphone	Linear	Ignore	1/x
Hydromorphone	Linear	Ignore	1/x
Codeine	Quadratic	Ignore	1/x
O-Desmethyltramadol	Quadratic	Ignore	1/x
Oxycodone	Quadratic	Ignore	1/x
6-Acetylmorphine	Quadratic	Ignore	1/x
Hydrocodone	Quadratic	Ignore	1/x
Tramadol	Quadratic	Ignore	1/x
Norbuprenorphine	Quadratic	Ignore	1/x
Fentanyl	Linear	Ignore	1/x
Buprenorphine	Linear	Ignore	1/x
Mitragynine	Linear	Ignore	1/x
Methadone	Linear	Ignore	1/x

Processed Sample Stability:

Room Temperature – 72 hours
Refrigerator – 72 hours

Notes:

Whole blood specimens may be diluted to achieve concentrations within the ranges of the standard curves for all analytes, except for Mitragynine. Mitragynine will be reported qualitatively (e.g., detected, identified).

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4.1.05 Title: CONFIRMATION – PHENCYCLIDINE IN BLOOD

Purpose and Scope: This procedure is to quantitatively determine phencyclidine in blood.

Principle: The deuterium labeled analog of each compound is added to each sample as an internal standard. The compounds and the deuterated internal standards are extracted from blood. The extracted analytes are then quantitated by GC/MS operated in SIM mode, using hydrogen as the carrier gas.

Materials:

- Co-polymer SPE columns, (6cc Cerex® Clin II 691-0506, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes*
- Autosampler vials, inserts, and TFE faced caps

*Other size tubes may be used as necessary.

Reagents:

Chemicals:

- Ethyl acetate
- Methanol
- 2-Propanol
- Ammonium hydroxide

Reagent solutions (Unless specified below, see Section 4.3 for preparation, QC, and storage instructions):

- Acetic acid, 1.0 M
- Phosphate buffer, 100 mM, pH 6.0
- Eluting Solution – ethyl acetate/2-propanol/ammonium hydroxide (90/6/4)
  - Prepare fresh daily for one time use.
  - QC: Check pH with pH paper.

Drug solutions (See Section 4.2 for preparation, QC, and storage instructions. See Section 6.3.1 for QC instructions):

- Calibration standard working solution level 1 – 1 µg/mL PCP
- Calibration standard working solution level 2 – 10 µg/mL PCP
- Control working solution level 1 – 1 µg/mL PCP
- Control working solution level 2 – 10 µg/mL PCP
- Internal standard working solution – 2 µg/mL PCP-D_5

Calibrators and Controls:

Calibrators are prepared in 1.0 mL aliquots at each of the concentrations listed below in labeled 16 x 125 mm glass tubes using negative whole blood and the specified calibration standard working solutions.

Controls are prepared in the same concentrations as calibrators. A control is run after the negative control, after every 10 samples, and at the end of the batch.

Calibrator / Control	Final Concentration of PCP (ng/mL)	Volume of PCP Working Solution Level 1 (1 µg/mL)	Volume of Whole Blood
1	10	10 µL	1000 µL
2	25	25 µL	1000 µL
3	50	50 µL	1000 µL
4	75	75 µL	1000 µL
Calibrator / Control	**Final Concentration of PCP (ng/mL)**	**Volume of PCP Working Solution Level 2 (10 µg/mL)**	**Volume of Whole Blood**
5	100	10 µL	1000 µL
6	250	25 µL	1000 µL
7	400	40 µL	1000 µL
8	500	50 µL	1000 µL
Negative Control	0	0	1000 µL

Preparation:

1. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Prepare calibrators and controls as described above.
3. Add 50 µL of PCP-D_5 internal standard (2 µg/mL) to each tube.
4. Add 4 mL of 100 mM Phosphate buffer (pH 6.0) to each tube and vortex.
5. Centrifuge each tube for at least 10 min at ~3000 rpm.

Solid Phase Extraction:

6. Place the Cerex Clin II SPE columns into an extraction manifold.
7. Load samples and run through SPE columns.
8. Wash the SPE columns as follows:
    a. 3 mL distilled water
    b. 1 mL 1.0 M acetic acid
    c. 3 mL methanol
9. Dry SPE columns for at least 15 minutes at ≥ 20 psi.

10. Place collection tubes in manifold under each SPE column and elute with 3 mL of ethyl acetate/2-propanol/ammonium hydroxide (90/6/4).
11. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40 ºC under a gentle stream of nitrogen.

Reconstitution:

12. Reconstitute with 100 µL ethyl acetate and vortex.
13. Transfer contents of each tube to an autosampler vial with insert. Cap and transfer to autosampler tray for GC/MS analysis.

GC/MS Analysis:

LVMPD Instrument	Tox #10 GCMS
Instrument Make/Model	Agilent 7890A GC and 5975C Mass Spectrometer
Software for Acquisition	Agilent ChemStation
Acquisition Method	PCP_B.m
Software for Data Analysis	Agilent MassHunter Quantitative Analysis
Data Analysis Method	PCP_B.m

GC/MS Parameters:

Inlet Liner	Splitless liner (RESTEK Gooseneck Splitless Liner #22406 or equivalent)
Column	DB-5MS, 20 m x 0.180 mm i.d. x 0.18 µm film thickness (or equivalent)
Injection Mode	Splitless mode
Injection Volume	0.5-1.0 µL
Injector Temperature	210ºC
GC Carrier Gas Flow	0.5 mL/min – constant flow mode
Oven Program	75ºC for 1 min, 35ºC/min to 300 ºC, hold at 300 ºC for 3 min
Thermal Aux 2	300 ºC
MS Source	Electron Impact (EI)
MS Source Temperature	230 ºC
MS Quad Temperature	150 ºC

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

Analyte	Quantifier Ion	Qualifier Ion
PCP-D_5	246	205
PCP	242	243, 200

Calibration Models

Analyte	Model Type	Origin	Weighting
PCP	Quadratic	Ignore	1/x

Processed Sample Stability:
Room Temperature – 24 hours
Refrigerator – 72 hours
Freezer – 72 hours

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4.1.06 Title: CONFIRMATION – BENZODIAZEPINES / Z-DRUGS IN BLOOD

Purpose and Scope: This procedure is intended to quantitatively determine 7-aminoclonazepam, zopiclone, zolpidem, zaleplon, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, flunitrazepam, temazepam, and diazepam in whole blood.

Principle: The deuterium labeled analogs of the target analytes are added to each sample as an internal standard. The analytes and the internal standards are extracted from whole blood using liquid-liquid extraction and analyzed by LC/MS/MS.

Materials:

- 16 x 100 mm glass screw-top culture tubes*
- Autosampler vials, inserts, and caps

*other sizes tubes may be used as necessary

Reagents:

Chemicals:

- Sodium Borate
- Water, LC Grade or higher
- Ethyl acetate, LC Grade or higher
- Acetonitrile, LC Grade or higher

Reagent Solutions (Unless specified below, see Chapter 4.3 for preparation, QC, and storage instructions):

- Saturated sodium borate buffer
- Water:ACN reconstitution solution

Drug Solutions (see Chapter 4.2 for preparation and storage instructions. See Section 6.3.1 for QC instructions):

- Internal standard working solution – 0.5 µg/mL flunitrazepam-D_7 / 1 µg/mL 7-aminoclonazepam-D_4, zopiclone-D_4, zolpidem-D_6, zaleplon-D_4, oxazepam-D_5, nordiazepam-D_5, clonazepam-D_4, lorazepam-D_4, alprazolam-D_5, temazepam-D_5, and diazepam-D_5 in methanol.
- Drug working solutions –
  Level 2 = 5 µg/mL flunitrazepam /10 µg/mL 7-aminoclonazepam, zopiclone, zolpidem, zaleplon, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, temazepam, and diazepam in methanol.
  Level 1 = 0.5 µg/mL flunitrazepam / 1 µg/mL 7-aminoclonazepam, zopiclone, zolpidem, zaleplon, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, temazepam, and diazepam in methanol.

Calibrators and Controls:

Calibrators are prepared in 1 mL aliquots of each of the concentrations listed below in labeled 16 x 100 mm glass screw-top culture tubes using negative whole blood and the specified drug working solution.

Controls are prepared in the same manner as calibrators. A control is run prior to case samples, after every 10 samples throughout the batch, and at the end of the run.

Calibrator / Control	BenzoZ Mix Final Concentration (ng/mL)	BenzoZ Mix Working Solution Level 1	Volume Whole Blood
1	5 / 10	10 µL	1000 µL
2	10 / 20	20 µL	1000 µL
3	25 / 50	50 µL	1000 µL
4	50 / 100	100 µL	1000 µL
Calibrator / Control	**BenzoZ Mix Final Concentration (ng/mL)**	**BenzoZ Mix Working Solution Level 2**	**Volume Whole Blood**
5	100 / 200	20 µL	1000 µL
6	200 / 400	40 µL	1000 µL
7	300 / 600	60 µL	1000 µL
Negative Control	0	0	1 mL

Preparation:

1. Pipet 1 mL of each casework blood specimen into labeled screw top tubes.
2. Prepare calibrators/controls as above.
3. Add 100 µL of internal standard to each and vortex.
4. Add 1 mL of saturated sodium borate buffer to each and vortex.
5. Add 4 mL of ethyl acetate to each.
6. Cap and rotate at least 2 minutes.
7. Centrifuge at least 2 minutes.
8. Transfer upper organic layer to labeled tubes.
9. Evaporate sample to dryness 35-40 °C under nitrogen.

Reconstitution:

10. Reconstitute in 200 µL of reconstitution solution (9:1 Water:ACN) and vortex
    Optional: Centrifuge at least 2 min.
11. Transfer the sample from the tube to an autosampler vial/insert, cap and transfer to autosampler tray for LC/MSMS analysis.

LC/MS/MS Analysis:

LVMPD Instrument	TOX #2 LCMSMS
Instrument Make/Model	Agilent 6420 Triple Quadrupole LC/MSMS
Software	Agilent MassHunter
Acquisition Method	BenzoZ_B.m
Data Analysis Method	BenzoZ_B.m
Reporting Method	BenzoZ_B.m

LC Parameters:

Multisampler Temperature	Room temperature	
Injection Volume	3-6 µL (default is 5 µL)	
Column	Poroshell 120 EC-C18 2.1mm x 75 mm 2.7 micron	
Column Temperature	35 °C	
Needle Wash	20 seconds	
Needle Wash Solution	1:1:1:1 Methanol:Water:IPA:ACN	
Mobile Phase A	0.1% Formic Acid in Water	
Mobile Phase B	0.1% Formic Acid in Acetonitrile	
Flow Rate	0.5 mL/min	
Gradient	Initial	10% B
	4 min	30% B
	8 min	40% B
	8.5 min	95% B
	10.5 min	95% B
	11 min	10% B

Post time 1.5 min

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

MSD Parameters:

Ionization	ESI
Polarity	Positive
Gas Temperature	330 °C
Gas Flow	11 mL/min
Nebulizer Pressure	35 psi
Capillary Voltage	3000 V

Analyte	Quantitation Transition	Qualifier Transition
7-Aminoclonazepam-D4	290.1 → 121.1	n/a
7-Aminoclonazepam	286.1 → 121.1	286.1 → 222.1
Zopiclone-D4	393.1 → 245.0	n/a
Zopiclone	389.1 → 245.0	389.1 → 217.1
Zolpidem-D6	314.2 → 235.1	n/a
Zolpidem	308.2 → 235.1	308.2 → 263.1
Zaleplon-D4	310.2 → 268.1	n/a
Zaleplon	306.1 → 236.1	306.1 → 264.1
Oxazepam-D5	292.1 → 246.1	n/a
Oxazepam	287.1 → 241.0	287.1 → 269.1
Nordiazepam-D5	276.1 → 140.0	n/a
Nordiazepam	271.1 → 208.0	271.1 → 165.0
Clonazepam-D4	320.1 → 274.1	n/a
Clonazepam	316.1 → 270.1	316.1 → 214.0
Lorazepam-D4	327.0 → 281.0	n/a
Lorazepam	321.0 → 229.0	323.0 → 229.0
Alprazolam-D5	314.1 → 286.1	n/a
Alprazolam	309.1 → 205.0	309.1 → 274.0
Flunitrazepam-D7	321.1 → 275.2	n/a
Flunitrazepam	314.1 → 268.1	314.1 → 239.1
Temazepam-D5	306.1 → 260.0	n/a
Temazepam	301.1 → 255.1	301.1 → 283.0
Diazepam-D5	290.1 → 154.0	n/a
Diazepam	285.1 → 193.1	285.1 → 91.1

Calibration Models:

Analyte	Model Type	Origin	Weighting
7-Aminoclonazepam	Quadratic	Ignore	1/x
Zopiclone	Linear	Ignore	1/x
Zolpidem	Quadratic	Ignore	1/x
Zaleplon	Linear	Ignore	1/x
Oxazepam	Quadratic	Ignore	1/x
Nordiazepam	Quadratic	Ignore	1/x
Clonazepam	Quadratic	Ignore	1/x
Lorazepam	Quadratic	Ignore	1/x
Alprazolam	Quadratic	Ignore	1/x
Flunitrazepam	Quadratic	Ignore	1/x
Temazepam	Quadratic	Ignore	1/x
Diazepam	Quadratic	Ignore	1/x

Processed Sample Stability:
Room Temperature – 72 hours
Refrigerator – 72 hours

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.1.07 Title: CONFIRMATION – CARISOPRODOL AND MEPROBAMATE IN BLOOD

Purpose and Scope: This procedure is to quantitatively determine carisoprodol and meprobamate in whole blood.

Principle: The deuterium labeled analog of each compound is added to each sample as an internal standard. The compounds and the deuterated internal standards are extracted from blood. The extracted analytes are then quantitated by GC/MS operated in SIM mode, using hydrogen as the carrier gas.

Materials:

- Co-polymer SPE columns, (6cc Cerex® Clin II 691-0506, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes with screw caps*
- GC/MS autosampler vials, inserts, and TFE faced caps

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled/purified water
- Hexane
- Ethyl acetate

Reagent Solutions (Unless specified below, see Chapter 4.3 for preparation, QC, and storage instructions):

- Acetic acid, 100 mM
- Phosphate buffer, 100 mM, pH 6.0
- 1/1 (v/v) Hexane / Ethyl Acetate. Prepare fresh daily for one time use.

Drug Solutions (see Chapter 4.2 for preparation and storage instructions. See Section 6.3.1 for QC instructions):

- Calibration standard working solution level 1 – 20 µg/mL CAR/MEP
- Calibration standard working solution level 2 – 100 µg/mL CAR/MEP
- Control working solution level 1 – 20 µg/mL CAR/MEP
- Control working solution level 2 – 100 µg/mL CAR/MEP

Internal standard working solution – 20 µg/mL CAR-D_7/MEP-D_7

Calibrators and controls:

Calibrators are prepared in 1 mL aliquots of each of the concentrations listed below in labeled 16 x 125 mm glass culture tubes using negative whole blood and the specified drug working solution.

Controls are prepared in the same concentrations as calibrators. A control is run prior to case samples, after every 10 samples throughout the batch, and at the end of the run.

Calibrator / Control	Final Concentration of CAR, MEP (ng/mL)	Volume of CAR, MEP Working Solution Level 1 (20 µg/mL)	Volume of Whole Blood
1	500	25 µL	1000 µL
2	1000	50 µL	1000 µL
3	2000	100 µL	1000 µL
Calibrator / Control	**Final Concentration of CAR, MEP (ng/mL)**	**Volume of CAR, MEP Working Solution Level 2 (100 µg/mL)**	**Volume of Whole Blood**
4	5000	50 µL	1000 µL
5	7500	75 µL	1000 µL
6	10000	100 µL	1000 µL
Negative Control	0	0	1000 µL

Preparation:

1. Pipet 1.0 mL of each casework blood specimen into a labeled 16 x 125 mm glass tube. *Note: casework sample dilutions are typical in order to obtain concentration values within the ranges of the calibration curves.*
2. Prepare calibrators and controls as described above.
3. Add 50 µL of internal standard working solution to each tube and vortex.
4. Add 4 mL of phosphate buffer, 100 mM, pH 6.0, to each tube and vortex.
5. Centrifuge each tube for 10 min at ~3000 rpm.

Solid Phase Extraction:

6. Place the Cerex Clin II SPE columns into an extraction manifold.
7. Load samples and run through SPE columns.
8. Wash SPE columns as follows:
    a. 3 mL distilled/purified water
    b. 1 mL acetic acid, 100 mM
9. Dry SPE columns for at least 20 minutes at ≥20 psi.
10. Add 2 mL hexane and aspirate.
11. Dry SPE columns for at least 5 minutes at ≥20 psi.
12. Place labeled 16 x 100 mm screw top glass culture tubes under SPE columns and elute with 3 mL of 1/1 (v/v) hexane/ethyl acetate.
13. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40°C under a gentle stream of nitrogen. Do not over dry.

Reconstitution:

14. Reconstitute with 100 µL ethyl acetate and vortex.
15. Transfer contents of each tube to an autosampler vial with insert. Cap and transfer to autosampler tray for GC/MS analysis.

GC/MS Analysis:

LVMPD Instrument	Tox #10 GCMS
Instrument Make/Model	Agilent 7890A GC and 5975C Mass Spectrometer
Software for Acquisition	Agilent ChemStation
Acquisition Method	CARMEP_B.m
Software for Data Analysis	Agilent MassHunter Quantitative Analysis
Data Analysis Method	CARMEP_B.m

GC/MS Parameters:

Inlet Liner	Split liner (RESTEK Low Pressure Drop Liner #21033 or equivalent)
Column	DB-5MS, 20 m x 0.180 mm i.d. x 0.18 µm film thickness (or equivalent)
Injection Mode	Split mode with 20:1 split ratio
Injection Volume	0.5-1.0 µL
Injector Temperature	250 ºC
GC Carrier Gas Flow	0.5 mL/min – constant flow mode
Oven Program	100 ºC for 0.5 min, 30º / min to 280 ºC, hold at 280 ºC for 4.5 min.
Thermal Aux 2	280 ºC
MS Source	Electron Impact (EI)
MS Source Temperature	230 ºC
MS Quad Temperature	150 ºC

Note: Slight variations in gradient may exist due to instrument capabilities, column properties, etc.

Analyte	Quantifier Ion	Qualifier Ion
MEP-D_7	151	121
MEP	144	114, 96
CAR-D_7	191	252
CAR	245	184, 158

Calibration Models:

Analyte	Model Type	Origin	Weighting
Meprobamate	Linear	Ignore	1/x
Carisoprodol	Linear	Ignore	1/x

Processed Sample Stability:
Room Temperature – 72 hours
Refrigerator – 72 hours
Freezer – 72 hours

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2 Title: CONFIRMATION – DRUG SOLUTION PREPARATIONS

Note: If alternate final volumes are desired, volumes may be revised providing the proportions are maintained. Variations to the formulations must be verified by another Forensic Scientist, Toxicology Supervisor, or Toxicology Manager. Verification is indicated on the Reagent Prep Log form in the "approved by" box.

Storage:
Unless otherwise noted, store all preparations in the freezer.

Expiration Date:
Expiration date is one year from date of preparation or earliest expiration/use by/retest/best before date of a component of the preparation, whichever is sooner.

Solvents:
Unless otherwise noted, methanol and acetonitrile used for preparations should be GC grade or better.

Quality Control:
See section **6.3.1 Quality Control Checks of Drug Stock and Working Solutions** for Quality Control procedures.

Amphetamines and Stimulants Blood Working Solutions

Note: The LVMPD Forensic Laboratory does not distinguish enantiomers of AMP, MDA, METH, MDMA, or MPH when reporting drug confirmation results.

Calibration Standard Working Solution Level 1 – 1 µg/mL AMP, MDA, METH, MDMA, PHEN, MPH

Amount	Ingredients	Measuring Device
1000 µL	Calibration Standard Working Solution Level 2 – 10 µg/mL AMP, MDA, METH, MDMA, PHEN, MPH	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol (*LC grade or better*)	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 2 – 10 µg/mL AMP, MDA, METH, MDMA, PHEN, MPH

Amount	Ingredients	Measuring Device
100 µL	1.0 mg/mL (±)-AMP Cerilliant standard (A-007-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL (±)-MDA Cerilliant standard (M-012-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL S(+)-METH Cerilliant standard (M-020-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL (±)-MDMA Cerilliant standard (M-013-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL PHEN Cerilliant standard (P-023-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL MPH Cerilliant standard (M-083-1ML)	Eppendorf Repeater pipette
QS to 10 mL	Methanol (*LC grade or better*)	10 mL serialized Class A volumetric flask

Control Working Solution Level 1 – 1 µg/mL AMP, MDA, METH, MDMA, PHEN, MPH
Prepare a 1 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 – 1 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH with the exception that the control materials must come from a different manufacturer than Cerilliant.

Control Working Solution Level 2 – 10 µg/mL AMP, MDA, METH, MDMA, PHEN, MPH
Prepare a 10 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH control working solution using a similar formulation as described for preparing the calibration standard working solution level 2 – 10 µg/mL AMP, MDA, METH, MDMA, PHEN, and MPH with the exception that the control materials must come from a different manufacturer than Cerilliant.

1 µg/mL AMP-D_{11}, MDA-D_5, METH-D_{11}, MDMA-D_5, PHEN-D_5, MPH-D_9 – Internal Standard Working Solution

Amount	Ingredients	Measuring Device
500 µL	100 µg/mL (±)-AMP-D_{11} Cerilliant standard (A-016-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL (±)-MDA-D_5 Cerilliant standard (M-010-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL (±)-METH-D_{11} Cerilliant standard (M-059-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL (±)-MDMA-D_5 Cerilliant standard (M-011-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL PHEN-D_5 Cerilliant standard (P-034-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL MPH-D_9 Cerilliant standard (M-127-1ML)	Eppendorf Repeater pipette
QS to 50 mL	Methanol	50 mL Class A volumetric flask

COC / CE / BZE Blood Working Solutions

Calibration Standard Working Solution Level 1 – 1 µg/mL COC, CE; 5 µg/mL BZE

Amount	Ingredients	Measuring Device
1 mL	Calibration Standard Working Solution Level 2 – 10 µg/mL COC,CE; 50 µg/mL BZE	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Acetonitrile (*LC grade or better*)	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 2 – 10 µg/mL COC, CE; 50 µg/mL BZE

Amount	Ingredients	Measuring Device
100 µL	1.0 mg/mL COC Cerilliant standard (C-008-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL CE Cerilliant standard (C-010-1ML)	Eppendorf Repeater pipette
500 µL	1.0 mg/mL BZE Cerilliant standard (B-004-1ML)	Eppendorf Repeater pipette
QS to 10 mL	Acetonitrile (*LC grade or better*)	10 mL serialized Class A volumetric flask

Control Working Solution Level 1 – 1 µg/mL COC, CE; 5 µg/mL BZE

Prepare a 1 µg/mL COC, CE; 5 µg/mL BZE control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 – 1 µg/mL COC, CE; 5 µg/mL BZE with the exception that the control materials must come from a different manufacturer than Cerilliant.

Control Working Solution Level 2 – 10 µg/mL COC, CE; 50 µg/mL BZE

Prepare a 10 µg/mL COC, CE; 50 µg/mL BZE control working solution using a similar formulation as described for preparing the calibration standard working solution level 2 – 10 µg/mL COC, CE; 50 µg/mL BZE with the exception that the control materials must come from a different manufacturer than Cerilliant.

Internal Standard Working Solution – 2 µg/mL COC-D_3, CE-D_3; 10 µg/mL BZE-D_3

Amount	Ingredients	Measuring Device
200 µL	100 µg/mL COC-D_3 Cerilliant standard (C-004-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL CE-D_3 Cerilliant standard (C-009-1ML)	Eppendorf Repeater pipette
1 mL	100 µg/mL BZE-D_3 Cerilliant standard (B-001-1ML)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Acetonitrile (*LC grade or better*)	10 mL Class A volumetric flask

Cannabinoids Blood Stock and Working Solutions
(silanized amber vials must be used)

Note: (-)-11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol (Cerilliant standard T-018) is used because it is also suitable for immunoassay. The LVMPD Forensic Laboratory does not distinguish between ± THCA when reporting drug confirmation results.

100 µg/mL THC Calibration Standard Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL (-)-Δ^9-THC Cerilliant standard (T-005-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 1 – 0.1 µg/mL THC, 11-OH-THC; 0.5 µg/mL THCA

Amount	Ingredients	Measuring Device
1 mL	Calibration Standard Working Solution – Level 2 – 1 µg/mL THC, 11-OH-THC; 5 µg/mL THCA	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 2 – 1 µg/mL THC, 11-OH-THC; 5 µg/mL THCA

Amount	Ingredients	Measuring Device
100 µL	100 µg/mL THC Calibration Standard Stock Solution	Eppendorf Repeater pipette
100 µL	100 µg/mL 11-OH-THC Cerilliant standard (H-026-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL (-)-11-nor-9-Carboxy-Δ^9-THC Cerilliant standard (T-018-1ML)	Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

100 µg/mL THC Control Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL (-)-Δ^9-THC control (e.g., Cayman #ISO60157 or Lipomed #THC-135-1LE)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

0.1 µg/mL THC, 11-OH-THC; 0.5 µg/mL THCA Control Working Solution Level 1
Prepare a 0.1 µg/mL THC, 11-OH-THC; 0.5 µg/mL THCA control working solution using a similar formulation as described for preparing the 0.1 µg/mL THC, 11-OH-THC; 0.5 µg/mL THCA calibration standard working solution, with the exception that the control material must come from a different manufacturer than Cerilliant.

1 µg/mL THC, 11-OH-THC; 5 µg/mL THCA Control Working Solution Level 2
Prepare a 1 µg/mL THC, 11-OH-THC; 5 µg/mL THCA control working solution using a similar formulation as described for preparing the 1 µg/mL THC, 11-OH-THC; 5 µg/mL THCA calibration standard working solution, with the exception that the control material must come from a different manufacturer than Cerilliant.

0.1 µg/mL THC-D_3, 11-OH-THC-D_3; 0.5 µg/mL THCA-D_3 Internal Standard Working Solution

Amount	Ingredients	Measuring Device
50 µL	100 µg/mL THC-D_3 Cerilliant standard (T-003-1ML)	Eppendorf Repeater pipette
50 µL	100 µg/mL 11-OH-THC-D_3 Cerilliant standard (H-041-1ML)	Eppendorf Repeater pipette
250 µL	100 µg/mL THCA-D_3 Cerilliant standard (T-004-1ML)	Eppendorf Repeater pipette
QS to 50 mL	Methanol	50 mL Class A volumetric flask

Narcotic Analgesics Blood Stock and Working Solutions
(silanized amber vials must be used)

10 µg/mL FEN Standard Stock Solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL FEN Cerilliant standard (F-002-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

100 µg/mL OXM Standard Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL OXM Cerilliant standard (O-004-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

100 µg/mL HYM Standard Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL HYM Cerilliant standard (H-004-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Narcotic Analgesics Standard Working Solution Level 1 – 0.01 µg/mL FEN; 0.05 µg/mL 6-AM, BUP, NBUP; 0.1 µg/mL OXM, HYM; 0.5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 1 µg/mL MTD

Amount	Ingredients	Measuring Device
1000 µL	Narcotic Analgesics Standard Working Solution Level 2 – 0.1 µg/mL FEN; 0.5 µg/mL 6-AM, BUP, NBUP; 1 µg/mL OXM, HYM; 5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 10 µg/mL MTD	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Narcotic Analgesics Standard Working Solution Level 2 – 0.1 µg/mL FEN; 0.5 µg/mL 6-AM, BUP, NBUP; 1 µg/mL OXM, HYM; 5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 10 µg/mL MTD

Amount	Ingredients	Measuring Device
100 µL	10 µg/mL FEN Standard Stock Solution	Eppendorf Repeater pipette
100 µL	100 µg/mL OXM Standard Stock Solution	Eppendorf Repeater pipette
100 µL	100 µg/mL HYM Standard Stock Solution	Eppendorf Repeater pipette
50 µL	100 µg/mL 6-AM Cerilliant standard (A-003-1ML)	Eppendorf Repeater pipette
50 µL	100 µg/mL BUP Cerilliant standard (B-902-1ML)	Eppendorf Repeater pipette
50 µL	100 µg/mL NBUP Cerilliant standard (N-912-1ML)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL HYC Cerilliant standard (H-003-1ML)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL TRM Cerilliant standard (T-027-1ML)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL ODT Cerilliant standard (T-035-1ML)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL OXC Cerilliant standard (O-002-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL MOR Cerilliant standard (M-030-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL COD Cerilliant standard (C-015-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL MG Cerilliant standard (M-152-1ML)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL (±)-MTD Cerilliant standard (M-007-1ML)	Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

10 µg/mL FEN Control Stock Solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL FEN control (e.g., Cayman #ISO60197)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL OXM Control Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL OXM control (e.g., Cayman #ISO60171 or Lipomed #M-406-FB-1LM)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL HYM Control Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL HYM control (e.g., Cayman #ISO60146 or Lipomed #M-407-FB-1LM)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL 6-AM Control Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL 6-AM control (e.g., Cayman #19418 or Lipomed #M-43-HC-1LM)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL BUP Control Stock Solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL BUP control (e.g., Cayman #ISO60178 or Lipomed #BUP-399-HC-1LM)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

Narcotic Analgesics Control Working Solution Level 1 – 0.01 µg/mL FEN; 0.05 µg/mL 6-AM, BUP, NBUP; 0.1 µg/mL OXM, HYM; 0.5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 1 µg/mL MTD

Prepare a 0.01 µg/mL FEN; 0.05 µg/mL 6-AM, BUP, NBUP; 0.1 µg/mL OXM, HYM; 0.5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 1 µg/mL MTD control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 - 0.01 µg/mL FEN; 0.05 µg/mL 6-AM, BUP, NBUP; 0.1 µg/mL OXM, HYM; 0.5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 1 µg/mL MTD, with the exception that the control material must come from a different manufacturer than Cerilliant.

Narcotic Analgesics Control Working Solution Level 2 – 0.1 µg/mL FEN; 0.5 µg/mL 6-AM, BUP, NBUP; 1 µg/mL OXM, HYM; 5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 10 µg/mL MTD

Prepare a 0.1 µg/mL FEN; 0.5 µg/mL 6-AM, BUP, NBUP; 1 µg/mL OXM, HYM; 5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 10 µg/mL MTD control working solution using a similar formulation as described for preparing the calibration standard working solution level 2 - 0.1 µg/mL FEN; 0.5 µg/mL 6-AM, BUP, NBUP; 1 µg/mL OXM, HYM; 5 µg/mL MOR, COD, OXC, ODT, HYC, TRM, MG; 10 µg/mL MTD, with the exception that the control material must come from a different manufacturer than Cerilliant.

Amount	Ingredients	Measuring Device
100 µL	10 µg/mL FEN Control Stock Solution	Eppendorf Repeater pipette
100 µL	100 µg/mL OXM Control Stock Solution	Eppendorf Repeater pipette
100 µL	100 µg/mL HYM Control Stock Solution	Eppendorf Repeater pipette
50 µL	100 µg/mL 6-AM Control Stock Solution	Eppendorf Repeater pipette
50 µL	100 µg/mL BUP Control Stock Solution	Eppendorf Repeater pipette
50 µL	100 µg/mL NBUP Control (e.g., Lipomed #BUP-982-FB-0.1LM)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL HYC Control (e.g., Cayman #ISO60144)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL TRM Control (e.g., Lipomed #TRA-779-HC-1LM)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL ODT Control (e.g., Lipomed #TRA-1523-HC-1LM)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL OXC Control (e.g., Lipomed #C-404-HC-1LM)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL MOR Control (e.g., Lipomed #M-35-FB-1LM)	Eppendorf Repeater pipette
50 µL	1.0 mg/mL COD Control (e.g., Cayman #ISO60140)	Eppendorf Repeater pipette
500 µL	100 µg/mL MG Control (e.g., Lipomed #MTR-1610-0.1LM)	Eppendorf Repeater pipette
100 µL	1.0 mg/mL (±)-MTD Control (e.g., Lipomed #MET-637-HC-1LM)	Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL FEN-D_5 Internal Standard Stock Solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL FEN-D_5 Cerilliant standard (F-001-1ML)	1 mL Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

Narcotic Analgesics Internal Standard Working Solution – 0.02 µg/mL FEN-D_5; 0.1 µg/mL 6-AM-D_6, BUP-D_4, NBUP-D_3; 0.2 µg/mL OXM-D_3, HYM-D_6; 1 µg/mL MOR-D_6, COD-D_6, OXC-D_6, ODT-D_6, HYC-D_6, TRM-^{13}C-D_3, MG-D_3, MTD-D_3

Amount	Ingredients	Measuring Device
40 µL	10 µg/mL FEN-D_5 Internal Standard Stock Solution	Eppendorf Repeater pipette
40 µL	100 µg/mL OXM-D_3 Cerilliant standard (O-003-1ML)	Eppendorf Repeater pipette
40 µL	100 µg/mL HYM-D_6 Cerilliant standard (H-049-1ML)	Eppendorf Repeater pipette
20 µL	100 µg/mL 6-AM-D_6 Cerilliant standard (A-026-1ML)	Eppendorf Repeater pipette
20 µL	100 µg/mL BUP-D_4 Cerilliant standard (B-901-1ML)	Eppendorf Repeater pipette
20 µL	100 µg/mL NBUP-D_3 Cerilliant standard (N-920-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL MOR-D_6 Cerilliant standard (M-085-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL COD-D_6 Cerilliant standard (C-040-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL OXC-D_6 Cerilliant standard (O-007-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL HYC-D_6 Cerilliant standard (H-047-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL MG-D_3 Cerilliant standard (M-182-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL cis-TRM-^{13}C, D_3 Cerilliant standard (T-029-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL ODT-D_6 Cerilliant standard (D-058-1ML)	Eppendorf Repeater pipette
200 µL	100 µg/mL (±)-MTD-D_3 Cerilliant standard (M-008-1ML)	Eppendorf Repeater pipette
QS to 20 mL	Methanol	20 mL Class A volumetric flask

Phencyclidine Blood Working Solutions

Calibration Standard Working Solution Level 1 – 1 µg/mL PCP

Amount	Ingredient	Measuring Device
1 mL	Calibration Standard Working Solution level 2 – 10 µg/mL PCP	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 2 – 10 µg/mL PCP

Amount	Ingredient	Measuring Device
100 µL	1.0 mg/mL PCP Cerilliant standard (P-007-1ML)	Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Control Working Solution Level 1 – 1 µg/mL PCP

Prepare a 1 µg/mL PCP control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 – 1 µg/mL PCP, with the exception that the control material must come from a different manufacturer than Cerilliant.

Control Working Solution Level 2 – 10 µg/mL PCP

Prepare a 10 µg/mL PCP control working solution using a similar formulation as described for preparing the calibration standard working solution level 2 – 10 µg/mL PCP, with the exception that the control material must come from a different manufacturer than Cerilliant.

Internal Standard Working Solution – 2 µg/mL PCP-D_5

Amount	Ingredients	Measuring Device
200 µL	100 µg/mL PCP-D_5 Cerilliant standard (P-003-1ML)	Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

Benzodiazepines/Z-Drugs Blood Working Solutions

Calibration Standard Working Solution Level 1 – 0.5 µg/mL FLU; 1 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ

Amount	Ingredients	Measuring Device
1 mL	Calibration Standard Working Solution Level 2 – 5 µg/mL FLU; 10 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 2 – 5 µg/mL FLU; 10 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ

Amount	Ingredients	Measuring Device
50 µL	1 mg/mL FLU Cerilliant standard (F-907-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL ZOP Cerilliant standard (Z-003-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL ZOL Cerilliant standard (Z-017-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL ZAL Cerilliant standard (Z-004-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL OXAZ Cerilliant standard (O-902-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL NORDIAZ Cerilliant standard (N-905-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL CLON Cerilliant standard (C-907-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL LORAZ Cerilliant standard (L-901-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL ALP Cerilliant standard (A-903-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL TEMAZ Cerilliant standard (T-907-1ML)	Eppendorf Repeater pipette
100 µL	1 mg/mL DIAZ Cerilliant standard (D-907-1ML)	Eppendorf Repeater pipette
1000 µL	100 µg/mL 7AC Cerilliant standard (A-915-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Control Working Solution Level 1 – 0.5 µg/mL FLU; 1 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ

Prepare a 0.5 µg/mL FLU; 1 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 – 0.5 µg/mL FLU; 1 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ, with the exception that the control material must come from a different manufacturer than Cerilliant.

Control Working Solution Level 2 – 5 µg/mL FLU; 10 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ

Prepare a 5 µg/mL FLU; 10 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 – 5 µg/mL FLU; 10 µg/mL 7AC, ZOP, ZOL, ZAL, OXAZ, NORDIAZ, CLON, LORAZ, ALP, TEMAZ, DIAZ, with the exception that the control material must come from a different manufacturer than Cerilliant.

0.5 µg/mL FLU-D_7; 1 µg/mL 7AC-D_4, ZOP-D_4, ZOL-D_6, ZAL-D_4, OXAZ-D_5, NORDIAZ-D_5, CLON-D_4, LORAZ-D_4, ALP-D_5, TEMAZ-D_5, DIAZ-D_5 Internal Standard Working Solution

Amount	Ingredients	Measuring Device
250 µL	100 µg/mL FLU-D_7 Cerilliant standard (F-915-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL 7AC-D_4 Cerilliant standard (A-917-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL ZOP-D_4 Cerilliant standard (Z-902-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL ZOL-D_6 Cerilliant standard (Z-001-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL ZAL-D_4 Cerilliant standard (Z-010-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL OXAZ-D_5 Cerilliant standard (O-901-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL NORDIAZ-D_5 Cerilliant standard (N-903-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL CLON-D_4 Cerilliant standard (C-905-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL LORAZ-D_4 Cerilliant standard (L-902-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL ALP-D_5 Cerilliant standard (A-902-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL TEMAZ-D_5 Cerilliant standard (T-902-1ML)	Eppendorf Repeater pipette
500 µL	100 µg/mL DIAZ-D_5 Cerilliant standard (D-902-1ML)	Eppendorf Repeater pipette
QS to 50 mL	Methanol	50 mL Class A volumetric flask

Carisoprodol and Meprobamate Blood Working Solutions

Calibration Standard Working Solution Level 1 – 20 µg/mL CAR, MEP

Amount	Ingredients	Measuring Device
2 mL	Calibration Standard Working Solution level 2 – 100 µg/mL CAR, MEP	Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Calibration Standard Working Solution Level 2 – 100 µg/mL CAR, MEP

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL CAR Cerilliant standard (C-077-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
1 mL	1.0 mg/mL MEP Cerilliant standard (M-039-1ML)	1 mL serialized Class A volumetric flask or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL serialized Class A volumetric flask

Control Working Solution Level 1 – 20 µg/mL CAR, MEP

Prepare a 20 µg/mL CAR, MEP control working solution using a similar formulation as described for preparing the calibration standard working solution level 1 – 20 µg/mL CAR/MEP, with the exception that the control materials must come from a different manufacturer than Cerilliant.

Control Working Solution Level 2 – 100 µg/mL CAR, MEP

Prepare a 100 µg/mL CAR, MEP control working solution using a similar formulation as described for preparing the calibration standard working solution level 2 – 100 µg/mL CAR/MEP, with the exception that the control materials must come from a different manufacturer than Cerilliant.

20 µg/mL CAR-D_7, MEP-D_7 Internal Standard Working Solution

Amount	Ingredients	Measuring Device
2 mL	100 µg/mL CAR-D_7 Cerilliant standard (C-083-1ML)	1 mL Class A volumetric flask (x 2) or an Eppendorf Repeater pipette
2 mL	100 µg/mL MEP-D_7 Cerilliant standard (M-131-1ML)	1 mL Class A volumetric flask (x 2) or an Eppendorf Repeater pipette
QS to 10 mL	Methanol	10 mL Class A volumetric flask

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4.3 Title: **CONFIRMATION – REAGENT PREPARATIONS**

Note: If alternate final volumes are desired, then weights and volumes may be revised providing the proportions are maintained. Variations to the formulations must be verified by another Forensic Scientist, Toxicology Supervisor, or Toxicology Manager. Verification is indicated on the Reagent Prep Log form in the "approved by" box.

Note: Depending on the weighing capacity of the analytical balance, additional decimal places may be recorded when weighing chemicals. The analyst should attempt to match the recipe weight as closely as possible without being under weight.

ACIDIC SOLUTIONS

Acetic acid, 1.0 M

- Add 5.76 mL of glacial acetic acid to distilled/purified water and dilute to 100 mL.
- QC: Check pH with pH paper. pH should be less than 7.
- Storage: Room temperature in glass container.
- Stability: 6 months.

Acetic acid, 100 mM

- Dilute 50 mL 1.0 M acetic acid to 500 mL with distilled/purified water.
- QC: Check pH with pH paper. pH should be less than 7.
- Storage: Room temperature in glass container.
- Stability: 6 months.

Acidic methanol, 1%

- Pipette 100 µL concentrated hydrochloric acid into a 10 mL volumetric flask and bring to volume with methanol.
- Prepare fresh daily for one time use.
- QC: Concurrently with use. All drug confirmation Batch Acceptance Criteria must be met for passing QC.

0.2% (v/v) Hydrochloric Acid in 2-Propanol

- To a 250 mL volumetric flask, add:
  - 500 µL concentrated hydrochloric acid, ACS grade or higher
  - Quantity sufficient to 250 mL with 2-propanol, LC grade or higher
- Swirl to mix.

- QC: Concurrently with use. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in glass container
- Stability: 6 months

BASIC SOLUTIONS

0.2 M Sodium Phosphate, Tribasic

- To a 2000 mL beaker, add:
  - 1000 mL distilled/purified water
  - 32.79 g sodium phosphate, tribasic, ACS grade or higher
- Mix using a stir bar and a stir plate.
- QC: Check pH with pH paper. pH should be basic.
- Storage: Room temperature in glass container
- Stability: 1 year

BUFFER SOLUTIONS

Phosphate buffer, 100 mM, pH 6.0

- Dissolve 1.70 g sodium phosphate dibasic and 12.14 g sodium phosphate monobasic in distilled/purified water and dilute to 1 liter. Adjust pH to 6.0 ± 0.1.
- QC: Check pH with pH paper.
- Storage: Refrigerate in glass container.
- Stability: 1 month. Inspect for contamination before use.

Sodium Borate Buffer, Saturated

- Dissolve 45 g sodium borate in 750 mL H2O while stirring with minimal heat on a hotplate. Cool completely before placing in storage jar.
- QC: Concurrently with use. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in glass container
- Stability: 6 months.

LC/MS/MS REAGENTS

50/50 Water/2-Propanol (Source Clean/Seal Pump Wash)

- Prepare a 50/50 mixture of LCMS Grade Water/LCMS Grade 2-Propanol in a plastic container (e.g., combine 200 mL of LCMS Grade Water and 200 mL of LCMS Grade 2-Propanol into a plastic container). Swirl to mix.
- QC: Not applicable. Reagent is used as a wash solution only.
- Storage: Room temperature in plastic container.

1:1:1:1 Methanol:Water:Acetonitrile:2-Propanol

- Prepare a 1:1:1:1 Methanol:Water:Acetonitrile:2-Propanol in a LCMS glass reagent bottle.
- For example:
  - To a 1000-mL LC reagent bottle, add:
    - 200 mL methanol, LC grade
    - 200 mL water, LC grade
    - 200 mL acetonitrile, LC grade
    - 200 mL 2-propanol, LC grade
- Swirl to mix.
- QC: Not applicable. Reagent is used as a wash solution only.
- Storage: Room temperature in glass container.

0.1% Formic Acid in Water (Mobile Phase)*

*This may be purchased or prepared in-house.

- To a 1000-mL LC reagent bottle, add:
  - 1 mL formic acid, LCMS grade
  - 1000 mL water, LCMS grade
- Swirl to mix.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in glass container.

0.1% Formic Acid in Acetonitrile (Mobile Phase)*

*This may be purchased or prepared in-house.

- To a 1000-mL LC reagent bottle, add:
  - 1 mL formic acid, LCMS grade
  - 1000 mL acetonitrile, LCMS grade
- Swirl to mix.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in glass container.

0.1% Formic Acid in Methanol

- To a 1000-mL LC reagent bottle, add:
  - 1 mL formic acid, LCMS grade

- 1000 mL methanol, LCMS grade
- Swirl to mix
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in glass container.

5 M Ammonium Formate

- Using an analytical balance, measure 6.30 g of ammonium formate. Add to water in 20 mL class A volumetric flask, bringing final volume to 20 mL.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Refrigerator in amber glass reagent vial.

Methanol with 5 mM Ammonium Formate and 0.1 % Formic Acid (Mobile Phase B)

- Prepare a 5 mM ammonium formate and 0.1% formic acid in a methanol solution in an LC/MS amber glass reagent bottle (e.g., using an Eppendorf Repeater pipette, add 1000 µL of 5 M ammonium formate and 1000 µL of formic acid to an LC/MS amber glass reagent bottle, GC/MS grade methanol to the 1000 mL mark of to the LC/MS amber glass reagent bottle). Swirl to mix.
- Alternatively can purchase premixed 0.1% Formic Acid in Methanol.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in LC/MS amber glass reagent bottle.

Water with 5 mM Ammonium Formate and 0.01% Formic Acid (Mobile Phase A)

- Prepare a 5 mM ammonium formate and 0.01% formic acid in a water solution in an LC/MS amber glass reagent bottle (e.g., using an Eppendorf Repeater pipette, add 1000 µL of 5 M ammonium formate and 100 µL of formic acid to an LC/MS amber glass reagent bottle, add LC/MS grade water to the 1000 mL mark of to the LC/MS amber glass reagent bottle). Swirl to mix.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in LC/MS amber glass reagent bottle.

(80:10:10) Acetonitrile:Isopropanol:Methanol (Needle Rinse for Narcotic Analgesics in Blood)

- Using an LC/MS amber glass reagent bottle for measuring, add 800 mL acetonitrile (LC/MS grade), 100 mL isopropanol (LC/MS grade) and 100 mL methanol (LC grade) to the LC/MS reagent bottle. Swirl to mix.
- QC: N/A
- Storage: Room temperature in LC/MS amber glass reagent bottle.

2% Formic Acid*

*This may be purchased or prepared in-house.

- Prepare a 2% formic acid in water solution in a 1000 mL class A volumetric flask (e.g., measure 20 mL of formic acid (LC/MS grade) using a 25 mL class A graduated cylinder, add to a 1000 mL class A volumetric flask and add water (distilled/purified or higher grade) at a quantity sufficient to 1000 mL). Plug flask and invert to mix.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Room temperature in LC/MS amber glass reagent bottle.
- Note: Commercially prepared reagent may be used in place of this recipe.

75:25 Methanol:Water

- To a 1000 mL LC reagent bottle, add:
  - 750 mL methanol, LC grade or higher
  - 250 mL water, LC grade or higher
- Swirl to mix.
- QC: N/A
- Storage: Room temperature in glass container

(90:10) Water:Isopropanol (Seal Pump Wash)

- Using an LC/MS glass reagent bottle for measuring, add 900 mL water (LC/MS grade) and 100 mL 2-Propanol (LC/MS grade). Swirl to mix.
- QC: N/A
- Storage: Room temperature in LC/MS glass reagent bottle.

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4.4 Title: BLANK WHOLE BLOOD PREPARATION

Negative blood is acquired from an approved vendor.

Reagents: Negative blood (e.g., porcine, bovine)
Deionized/distilled water
Sodium fluoride
Potassium oxalate

Preparation: Prepare an approximate 9:1 mixture of blood:deionized/distilled water solution containing sodium fluoride (approximately 10.0 mg/mL of blood) and potassium oxalate (approximately 2.0 mg/mL of blood).

1. Combine all reagents into a plastic container with a lid (see table below). Mix thoroughly.

Note: Sodium fluoride and potassium oxalate can be mixed with the deionized/distilled water prior to adding to the blood.

2. Label the prepared blank blood.
3. The blank blood should be transferred to smaller labelled containers for storage.
4. QC: See Chapter 5.6 and Chapter 6 section 6.5.1.1.
5. Storage: Freezer
6. Expiration date: 1 year (frozen)
   30 days (thawed)

Use the following table as a guideline. Weights and volumes are approximate. Other amounts may be prepared with prior calculation verification by a second member of the Toxicology Detail.

Sodium Fluoride (g)	Potassium Oxalate (g)	Water (mL)	Porcine Blood (mL)
9.0	1.8	100	900
30.0	6.0	333	3000
35.0	7.0	390	3500

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5.0 Title: ETHANOL ANALYSIS BY DUAL COLUMN GC HEADSPACE

Purpose and Scope

The purpose of this procedure is to determine the quantitative amount of ethanol (ethyl alcohol) in whole blood and urine.

Principle

The method relies on the principle described by Henry's Law which states that at a constant temperature, the volatile components in a solution will enter into a state of equilibrium between the liquid and vapor phases. Duplicate aliquots of blood and internal standard are thermally equilibrated and the resulting vapor is sampled and transferred onto two capillary columns contained within the gas chromatograph. The capillary columns separate the volatiles and the volatiles pass individually through the flame-ionization detectors (FIDs). Quantification of ethanol is achieved by the comparison of the ratio of detector response of ethanol in each sample to that of the internal standard, with the resulting ratio being compared to the standard curve.

Instrumentation

The instrumentation used for the analysis is a Perkin Elmer TurboMatrix HS110 with Clarus 500 or Clarus 580 model gas chromatograph operated as a dual column instrument with flame-ionization detectors and a headspace sample inlet. An Elite-BAC-1 Advantage fused silica column (30m, ID 0.32 mm, DF 1.8 – Perkin Elmer Cat. # N9315071, or equivalent) and an Elite-BAC-2 Advantage fused silica column (30m, ID 0.32 mm, DF 0.6 – Perkin Elmer Cat. # N9315073, or equivalent) are used. A copy of the instrument parameters is located within the method validation documentation.

Method Validation

The ethanol analysis by GC Headspace method was subject to a validation procedure following Scientific Working Group of Forensic Toxicology (SWGTOX) standard practices. Method validation documentation is kept in Qualtrax. Substantial changes in the analysis will require a re-validation of the method.

Materials

- Headspace vials (20mm, 22mL, crimp top or equivalent)
- Crimp seals (20mm PTFE/Rubber or equivalent)
- Vial seal crimper

- Diluter/Dispenser (Hamilton 500 or 600 series or equivalent)
- Absorbent wipes

Reagents (unless otherwise noted, store per manufacturers' requirements)

- Certified aqueous ethanol standards (10 mg/dL, 20 mg/dL, 80 mg/dL, 200 mg/dL and 400 mg/dL)
- Certified aqueous ethanol controls (at least three levels 0.02 - 0.40 g/dL)
- (e.g., 0.020 g/dL, 0.150 g/dL and 0.400 g/dL)
- Internal standard (0.015% v/v aqueous solution of 1-propanol. Store at room temperature)
- Negative whole blood control
- Positive whole blood ethanol control (60 – 100 mg/dL)
  (e.g., 80 mg/dL)
- Negative urine control (when applicable)
- Positive urine ethanol control (when applicable) (60 – 100 mg/dL)
  (e.g., 80 mg/dL)
- Mixed volatile resolution check sample containing acetaldehyde, acetone, methanol, ethanol and 2-propanol

Procedure

Preparing Samples:

An analyst will use the same lot number of internal standard and the same diluter/dispenser when preparing samples for casework, standards and/or controls in a single day. Casework samples will be pipetted in duplicate. All samples (standards, controls and casework samples) will be allowed to come to room temperature before pipetting.

Urine DUI samples are quantitatively tested only if they are collected from subjects with hemophilia or other medical conditions as described in NRS 484C.160 #4. For urine ethanol quantitation, the subject must void the bladder fully, and then collection of a second voiding at least 20 minutes later may be used for testing. Procedures for urine analysis will be the same as those for blood, with the exception that a positive urine control and a negative urine control must be analyzed in the batch.

The process for preparing whole blood casework samples for analysis is described below. Standards and controls are prepared using the same sampling methodology.

1) Prepare a Sequence Table and label headspace vials with vial position and Lab number. Item number should be used for cases with multiple subjects, and item number and draw time should be used for cases with multiple draws.
2) Open one blood kit at a time.
3) Remove one blood tube from the kit and label with Lab number/item number and analyst's initials.
4) Invert the blood tube several times and/or vortex to re-suspend the blood cells.

5) Compare the Lab number/item number on the blood tube label with the Lab number/item number on the headspace vials.
6) Using the diluter, aspirate 100 µL of sample and dispense with 1000 µL of internal standard solution into the appropriately labeled headspace vial. Both duplicate aliquots will be pipetted at this time.
7) Flush the diluter tube as necessary between duplicate aliquots and at least twice between each case sample. Wipe withdraw tip with absorbent wipe between sampling and dispensing.
8) Cap the vials and crimp securely. A securely crimped cap should not rotate on the vial.
9) Return the blood tube to its respective blood kit.
10) Place the vials in the TurboMatrix magazine, verifying the location of each sample.

Preparing Sample Dilutions:

If the BAC/UAC > 0.400 g/100mL, the sample may be diluted to obtain a value within the range of the standard curve. A 1:2 dilution should be adequate for most cases. The process to obtain a 1:2 dilution of a sample is described below.

A 1:2 dilution of a sample is achieved as follows:
1) Change the dispensing parameters on the Diluter/Dispenser to 50 µL for the specimen and 500 µL for the internal standard.
2) Prime the Diluter/Dispenser by flushing several times.
3) Using the diluter, aspirate 50 µL of negative whole blood (use negative urine if applicable) and dispense 500 µL of internal standard solution into the labeled headspace vial. Both duplicate aliquots will be pipetted at this time.
4) Flush the diluter tube as necessary between duplicate aliquots and at least twice between each case sample. Wipe withdraw tip with absorbent wipe between sampling and dispensing.
5) Using the diluter, aspirate 50 µL of the sample and dispense 500 µL of internal standard solution into the labeled headspace vial which already contains 50 µL of negative whole blood and 500 µL of internal standard. Both duplicate aliquots will be pipetted at this time.
6) Flush the diluter tube as necessary between duplicate aliquots and at least twice between each case sample. Wipe withdraw tip with absorbent wipe between sampling and dispensing.
7) Cap the vials and crimp securely. A securely crimped cap should not rotate on the vial.
8) Return the blood tube to its respective blood kit.
9) Place the vials in the TurboMatrix magazine, verifying the location of each sample.
10) Change the multiplier factor to 2 on the blood ethanol sequence to account for the 1:2 dilution.
11) Change the dispensing parameters on the Diluter/Dispenser to 100 µL for the specimen and 1000 µL for the internal standard.

NOTE: The Dilutor/Dispenser will be checked at the relevant measurements (e.g., 50 µL and 500 µL) prior to performing sample dilutions. The requirements for checking the

Dilutor/Dispenser are located in the section titled “Appendix - Quality Control Plan”. Pipettes are not to be used for performing sample dilutions for ethanol analysis.

Instrument Sequences

Standard Curve Sequence

1 0.02 g/100mL Aqueous Standard
2 0.08 g/100mL Aqueous Standard
3 0.20 g/100mL Aqueous Standard
4 0.40 g/100mL Aqueous Standard
5 0.01 g/100mL Aqueous LOD Check

Example Sequence for a Whole Blood Ethanol Batch of 5 Samples

1 0.02 g/100mL Aqueous Control
2 0.15 g/100mL Aqueous Control
3 0.40 g/100mL Aqueous Control
4 Internal Standard n-Propanol Blank
5 Positive Whole Blood Control
6 Mixed Volatile Resolution Check
7 Negative Whole Blood Control
8 Sample A
9 Sample A
10 Sample B
11 Sample B
12 Sample C
13 Sample C
14 Sample D
15 Sample D
16 Sample E
17 Sample E
18 0.05 g/100mL Aqueous Control

Batch Acceptance Requirements:

Except as noted below, each channel is treated independently when assessing batch acceptance requirements.

Calibration:

Ethanol solutions of 20 mg/dL, 80 mg/dL, 200 mg/dL and 400 mg/dL are used to establish a linear calibration curve. A linear calibration curve must be established each day by each analyst on the utilized instrument prior to running casework samples. Ethanol results of each calibration standard must be no greater than ± 5% of the target value (for ethanol concentrations < 0.05 g/100mL, results must be no greater than ± 0.005 g/100mL), as

calculated on the Fit Analysis Output printout. A correlation of determination (r^2) value greater than or equal to 0.995 must be achieved before casework samples may be analyzed.

Limit of Detection (LOD) Check:

A positive aqueous ethanol standard of 10 mg/dL will be run with each batch to ensure that the instrument can detect ethanol at the administratively defined LOD. The LOD check can be from the same or different source than the calibration standards. To be acceptable, the computer software must identify the ethanol peak at a concentration less than 0.02 g/100mL in both channel A and channel B chromatograms.

1-Propanol Blank:

A blank consisting of only internal standard must be analyzed before casework samples in each batch. The 1-propanol blank must result in one peak consistent with 1-propanol.

Positive Whole Blood Control:

When running a batch for blood ethanol analysis, a positive whole blood control with an ethanol value between 60 – 100 mg/dL must be analyzed before casework samples in each batch. Whole blood controls must be no greater than ± 0.005 g/100mL of the calculated mean value (for ethanol concentrations > 0.100 g/100mL, results must be no greater than ± 10% of the calculated mean value).

Positive Urine Control:

When running a batch for urine ethanol analysis, a positive urine control with an ethanol value between 60 – 100 mg/dL must be analyzed before casework samples in each batch. Urine controls must be no greater than ± 0.005 g/100mL of the calculated mean value (for ethanol concentrations > 0.100 g/100mL, results must be no greater than ± 10% of the calculated mean value).

Mixed Volatile Resolution Check:

A mixed volatile resolution check consisting of five target components (acetaldehyde, acetone, methanol, ethanol and 2-propanol) must be analyzed in order to demonstrate the resolution of these components. The qualitative mixed volatile resolution check must be analyzed before casework samples in each batch and result in the resolution of the five components. If a target component peak is not named by the software, the retention time of the peak must be no greater than ± .05 seconds of the retention time from the previous run.

Negative Whole Blood Control:

When running a batch for blood ethanol analysis, a negative whole blood control (no ethanol) must be analyzed before casework samples in each batch. The negative whole blood control result must have an ethanol value of “none detected” based on reporting protocols below.

Negative Urine Control:

When running a batch for urine ethanol analysis, a negative urine control (no ethanol) must be analyzed before casework samples in each batch. The negative urine control result must have an ethanol value of "none detected" based on reporting protocols below.

Positive Aqueous Ethanol Controls:

Positive aqueous ethanol standards from a source different than the calibration standards will be used as controls. These controls will have ethanol values of 0.02-0.40 g/dL and will be analyzed before casework samples in each batch. In addition, a control will be run once every five cases (10 (5x2) samples) and the last specimen of a batch will be a control. Concentrations of positive aqueous ethanol controls of 0.050 g/dL to 0.400 g/dL must be no greater than ± 5% of their target value. For ethanol concentrations < 0.05 g/100mL, results must be no greater than ± 0.005 g/100mL.

If one positive aqueous ethanol control does not pass quality control requirements, ethanol results for casework samples in the batch that are bracketed by controls meeting quality control requirements are valid and can be reported. Casework samples that are bracketed by the failed control must be repeated. If more than one positive aqueous ethanol control does not pass quality control requirements, all samples in the batch must be repeated.

NOTE: If any of the batch acceptance requirements listed above are not met, all casework samples/vials in the batch must be re-analyzed. It is noted that even though all acceptance criteria are met within a batch, the ethanol analyst must rely on their training and experience to determine if any anomalies exist that do not fall into the categories discussed below. In these instances the analyst should discuss the anomaly/anomalies with the Toxicology Manager or Supervisor in order to determine if all or part of a batch should be repeated to ensure that the reported results are accurate. If the decision is made to repeat all or part of a batch, the discussion should be documented in the case file. If a batch is aborted due to the reasons listed above, casework samples/vials which have not been heated during the thermostat segment of the procedure may be analyzed in the next batch without re-pipetting. However, all samples/vials to be used in the next batch will have been pipetted on the same day. Any vial that has been heated during the thermostat segment in a failed batch cannot be used in any subsequent batch. Samples may be refrigerated overnight and run on the instrument the following day. All samples prepared but not run on the instrument by the following day must be re-pipetted.

Casework Replicate Sample Requirements

Four quantitative results expressed to the fourth decimal place are compared. The highest result must be no greater than± 5% of the lowest result for 0.0500 g/100mL ≤ BAC/UAC ≤ 0.4000 g/100mL. For 0.0200 g/100mL ≤ BAC/UAC < 0.0500 g/100mL, the highest result must be no greater than ± 0.0050 g/100mL of the lowest result. For BAC/UAC < 0.0200 g/100mL, the previously stated requirements do not apply. Standard rules of rounding apply.

Reporting

The reported ethanol concentration is obtained by truncating the average of the four quantitative results to the 3rd decimal place. If 0.010 g/100mL ≤ BAC/UAC < 0.020 g/100mL, report “a concentration of alcohol of less than 0.020 g/100ml of blood”. If the BAC/UAC < 0.010 g/mL, report “none detected". If the result is above the highest ethanol standard used to calibrate the instrument, report greater than the highest standard (i.e., “a concentration of alcohol of greater than 0.400 g/100ml of blood”).

Sample Dilution Reporting: When using a sample dilution, the calculated BAC/UAC value is reported when the concentration of ethanol falls within the range of the standard curve (0.020 – 0.400 g/100mL) prior to applying the multiplier factor. For example, if a calculated BAC = 0.554 g/100mL was obtained using a 1:2 dilution, divide 0.554 g/100mL by the multiplier factor 2. The calculated value (0.277 g/100mL) falls within the range of the standard curve. Therefore, the BAC is reported as 0.554 g/100mL.

Trace Ethanol Reporting Exception: When the BAC/UAC < 0.020 g/100 mL and the instrument identifies trace amounts of ethanol in one or more of the four results (i.e., 0.010 g/100mL ≤ BAC/UAC < 0.020 g/100mL) but does not identify ethanol in one or more of the other results (i.e., BAC/UAC = 0.000 g/100mL), report “none detected".

Deviations from the reporting protocol outlined above must be approved by the Toxicology Manager/Designee.

Measurement Uncertainty

Measurement uncertainty is reported for all quantitative results that fall within the range of the standard curve (0.020 g/100mL ≤ BAC/UAC ≤ 0.400 g/100mL), including sample dilution results that fall within the range of the standard curve prior to applying the multiplier factor. Standard rules of rounding are used to calculate measurement uncertainty results to the thousandth decimal place.

Note: The LVMPD administratively defines the uncertainty of measurement for low ethanol concentrations (i.e., 20 mg/dL ≤ Ethanol < 50 mg/dL) to be +/- 5 mg/dL.

Measurement uncertainty documents are located in Qualtrax at Documents\LVMPD\Forensic Lab\Toxicology\Measurement Uncertainty. The measurement uncertainty will be reviewed and/or recalculated every two years and will be recalculated if there are procedural changes to the method that affect the quantitative measurement. The measurement uncertainty may be reviewed and/or recalculated at any time at the discretion of the Toxicology Manager.

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5.1 Title: BLOOD ETHANOL CALCULATIONS (RETROGRADE EXTRAPOLATION, ANTEROGRADE EXTRAPOLATION, AND THE WIDMARK EQUATION)

Purpose and Scope

This procedure is intended to provide guidance for performing blood ethanol calculations involving retrograde and anterograde extrapolation and the use of the Widmark Equation. Because each case will contain different scenarios and information, the forensic scientist must rely on his or her training and experience to determine if and how the calculation will be performed. This procedure may also be used to estimate breath alcohol concentrations.

Principle

At blood alcohol concentrations (BACs) greater than 0.02 g/100 mL, the elimination of ethanol from the body follows zero-order kinetics, that is, alcohol is eliminated from the body at a constant rate per period of time.[1] Therefore it is possible to estimate the BAC of an individual at a time prior to or after the blood draw if certain information is provided.

Note: At concentrations of approximately 0.01-0.02 g/100mL zero-order kinetics no longer apply. The elimination rate changes from zero-order to first-order kinetics. Retrograde extrapolation should not be performed when the BAC is 0.02 g/100 mL or less.

Retrograde Extrapolation

Retrograde extrapolation is the process of estimating an individual's blood alcohol concentration at some time prior to the time the specimen was obtained for analysis.

Retrograde extrapolation equation:

$BAC_{time\ prior} = BAC_{blood\ draw} + \beta t$

- $BAC_{time\ prior}$ - BAC at a time prior to the blood draw
- $BAC_{blood\ draw}$ - BAC at time of blood draw
- β - Elimination rate
- t - time between driving and blood draw

Retrograde Extrapolation Procedure:

- Determine if the subject was in the absorptive or post-absorptive phase. Retrograde extrapolation can only be determined if the subject was in the post-absorptive phase at the time of interest ($BAC_{time\ prior}$).
- $BAC_{blood\ draw}$ and time of the blood draw and the incident must be known.
- Use appropriate ethanol elimination rate(s).
- State your assumptions.
- Decide whether your answer will be expressed as "at least" or as a range.

- Retrograde extrapolation is only an ESTIMATION. Answers should not be given to three decimal places.
- Retrograde extrapolation should not be performed when the blood alcohol concentration is 0.02 g/100 mL or less.
- If the amount or type of information that has been provided to you is not sufficient, do not perform the calculation.

Response Examples:

- Assuming an average elimination rate and that the subject was in the post-absorptive phase at the time of the incident, I would estimate her BAC to be at least 0.22 g/100 mL at 2 AM.
- I would not feel comfortable estimating the blood alcohol concentration of the subject at 11 PM, the time of driving, because I believe that the subject was still in the absorptive phase at that time.

Widmark Equation

The Widmark equation is used to perform anterograde extrapolation and to estimate the number of drinks in an individual's system. It assumes that absorption and distribution of the entire dose of ethanol is complete and that first-pass metabolism is negligible

Widmark equation[1]:

$A = r \times p \times C$

- A - Amount of ethanol in grams
- r - Widmark's rho factor
  - rho (Men) = 0.68 (SD ± 0.085, CV 13%, range 0.55 to 0.86)
  - rho (Women) = 0.55 (SD ± 0.055, CV 10%, range 0.47 to 0.65)
- p - Body weight in kilograms
- C - Blood alcohol concentration in g/100 mL

Response Example:

- I would estimate that at least 3 standard drinks would need to be in the subject's system in order to register a BAC of 0.125 g/100 mL.

Anterograde Extrapolation

Anterograde extrapolation is the process of estimating an individual's blood alcohol concentration at a time after drinking. It may be used with the Widmark equation to determine the number of drinks in an individual's system.

Anterograde Extrapolation Equation:

$BAC_t = BAC_o - \beta t$

- BAC_t - BAC at time of interest

- BAC_o - BAC if all drinks have been absorbed, no elimination
- β - Elimination rate
- t - Number of hours between the time of interest and start of drinking

Anterograde Extrapolation Procedure:

- Determine if the subject was in the absorptive or post-absorptive phase at the time of interest. Anterograde extrapolation can only be determined if the subject was in the post-absorptive phase.
- Time the subject started drinking and time of interest, and number, type, volume, and % v/v alcohol of drinks must be given in the hypothetical.
- Use appropriate ethanol elimination rate(s).
- State your assumptions.
- Decide whether your answer will be expressed as “at least” or as a range.
- Anterograde extrapolation is only an ESTIMATION. Answers should not be given to three decimal places.
- If the amount or type of information that has been provided to you is not sufficient, do not perform the calculation.

Response Examples:

- Assuming an average elimination rate and that the subject was in the post-absorptive phase at 1 AM, the time of driving, I would estimate the subject’s BAC to be between 0.09 and 0.11 g/100 mL.
- Assuming an average elimination rate and that the subject was in the post-absorptive phase at 3 AM, I would estimate that the subject consumed at least 5 standard drinks.
- Based on this hypothetical, I would not feel comfortable estimating the subject’s BAC at 10:45 PM because I believe that the subject was still in the absorptive phase.

References

The following are some things to be considered when performing ethanol calculations. For more information see Chapter 3 in *Garriott's Medicolegal Aspects of Alcohol*.

Time to Reach Peak BAC after end of drinking[1]

- Empty Stomach 30 - 60 minutes
- With Food 45 - 90 minutes
- Extraneous Circumstances 90 - 120 minutes

Mean Ethanol Elimination Rates[2-3]

- Healthy adults
  - Men 0.015 g/100mL/hr
  - Women 0.018 g/100mL/hr
- DUI drivers 0.019 g/100mL/hr
- Alcoholics 0.023 g/100 mL/hr
- Exceptions Slower elimination rates for Eskimos, American Indians, Asians

In moderate drinkers 0.015 g/100mL/hr has been found to be a good average elimination rate value.[3]

Standard drink[4]

- Beer - 12 fl oz (5% alcohol)
- Wine - 5 fl oz (12% alcohol)
- Distilled spirit - 1.5 fl oz (80 proof - 40% alcohol)

Conversion Factors

1 fl oz = 29.6 mL
1 kg = 2.2 lb
Density of ethanol = 0.789 g/mL at room temperature

[1]Garriott's Medicolegal Aspects of Alcohol, 5th edition, Y.H. Caplan and B.A. Goldberger, Lawyers & Judges Publishing, 2009

[2]CCI Forensic Interpretation of Alcohol, R. B. Forney, Jr.'s lecture, 2011.

[3]Jones, A.W. Evidence-based survey of the elimination rates of ethanol from blood with applications in forensic casework. *Forensic Sci Int.* 200(1-3):1-20, 2010.

[4] http://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/what-standard-drink

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5.2 Title: 1-PROPANOL INTERNAL STANDARD PREPARATION

Procedure

A. Purpose: 1-propanol is used as an internal standard in the quantitative analysis of ethanol.

B. Materials: 5000 mL Class A volumetric flask
Pipette(s) suitable for measuring 1.5 mL
Container large enough to hold 10 liters

C. Reagents: 1-propanol, ACS grade or better
Distilled/purified Water

D. Preparation of internal standard working solution: 0.015 % (v/v) 1-propanol

1. Pipette 1.5 mL of 99.9 % 1-propanol (e.g., Alfa Aesar 41465) into a 5000 mL Class A volumetric flask.

2. Add distilled/purified water to the 5000 mL Class A volumetric flask to achieve a final volume of 5000 mL.

3. Place the solution in a container large enough to hold 10 liters.

4. Using a 5000 mL Class A volumetric flask, add an additional 5000 mL of distilled/purified water to the container.

5. Mix the internal standard working solution.

6. Label the container.

7. Transfer to labeled reagent bottles.

8. Expiration date is one year from date of preparation or earliest expiration/use by/retest date of a component of the preparation, whichever is sooner. Store at room temperature. Discard if it becomes turbid or moldy.

9. QC using Headspace GC-FID as outlined in section *5.6 Ethanol Analysis Quality Assurance*.

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5.3 Title: MIXED VOLATILE RESOLUTION CHECK

Procedure

A. Purpose: This reagent is used to demonstrate the resolution of methanol, ethanol, isopropanol, acetaldehyde, and acetone in the quantitative analysis of blood ethanol by headspace GC.

B. Materials: 100 mL volumetric flask
Pipette(s) suitable for measuring 50 µL – 127 µL

C. Reagents: Methanol, GC grade
Ethanol, absolute, 200 proof
Isopropanol (2-propanol)
Acetaldehyde
Acetone
Distilled/purified Water

D. Preparation

1. Deliver to a 100mL volumetric flask:

126 µL methanol	50 µL acetaldehyde
127 µL ethanol	50 µL acetone
127 µL isopropanol	

2. Dilute to the fiduciary mark with distilled/purified water.

3. Transfer to a labeled container.

4. Expiration date is one year from date of preparation or earliest expiration/use by/retest date of a component of the preparation, whichever is sooner. Store refrigerated. Protect from light.

5. QC using Headspace GC-FID as outlined in section *5.6 Ethanol Analysis Quality Assurance*.

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5.4 Title: POSITIVE WHOLE BLOOD ETHANOL CONTROL

Procedure

A. Purpose: A positive whole blood ethanol control is the matrix matched positive control used for whole blood ethanol analysis. This procedure describes the process for preparing an in house positive whole blood ethanol control. The positive whole blood ethanol control is typically purchased from a vendor.

B. Materials: 20 mL Class A Volumetric Flask
Analytical Balance (e.g., Tox Balance #3) or appropriate pipette

C. Reagents: Absolute Ethanol (100% Ethanol), ACS grade or better
Negative Whole Blood

D. Prepare a positive whole blood ethanol control as follows:

1) Add approximately 10 mL of negative whole blood to a 20 mL Class A volumetric flask.

2) If the control is being prepared using ethanol weight, place the 20 mL Class A volumetric flask on an analytical balance (e.g., Tox Balance #3) and tare the balance.

3) Drop by drop, add absolute ethanol to the 20 mL Class A volumetric flask. Listed below are a few examples relating target ethanol concentration and the target weight/volume of ethanol. Note that the amount of measured ethanol does not need to be exact.

Target Ethanol Concentration (g/100mL)	Target Ethanol Weight (g)	Target Ethanol Volume (µL)
0.060	0.0120	15.2
0.080	0.0160	20.3
0.100	0.0200	25.3

4) Record the weight or volume of absolute ethanol added to the 20 mL Class A volumetric flask on the Toxicology Reagent Preparation Log.

5) Add negative whole blood to the 20 mL Class A volumetric flask to the fiduciary mark to achieve a final volume of 20 mL. Mix the solution.

6) Calculate the concentration of ethanol using the appropriate formula:

By Weight

$$\frac{\text{grams of ethanol}}{20\ \text{mL}} \times 100\ \text{mL} = \frac{\text{x grams ethanol}}{100\ \text{mL}}$$

Sample calculation when 0.0145 g of ethanol are measured:

(0.0145 g ethanol)/(20 mL) x (100 mL) = (0.073 g ethanol/100 mL)

By Volume

$$\frac{\mu\text{L of ethanol}}{20\ \text{mL}} \times \frac{0.789\ \text{g ethanol}}{\text{mL}} \times \frac{1\ \text{mL}}{1000\ \mu\text{L}} \times 100\ \text{mL} = \frac{\text{x grams ethanol}}{100\ \text{mL}}$$

7) Record the ethanol concentration to three decimal places. Standard rules of rounding apply.

8) Transfer the reagent to labeled containers (typically 2 mL per container). Store in a refrigerator or freezer. When stored in the freezer, the expiration date is one year from date of preparation or earliest expiration/use by/retest date of a component of the preparation, whichever is sooner. When thawed and stored in a refrigerator, this reagent has a 30 day expiration date from the date of thawing or the expiration date or frozen preparation, whichever is sooner.

9) Experimentally determine the ethanol concentration using Headspace GC-FID as outlined in section *5.6 Ethanol Analysis Quality Assurance*.

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5.5 Title: POSITIVE URINE ETHANOL CONTROL

Procedure

A. Purpose: A positive urine ethanol control is the matrix matched positive control used for urine ethanol analysis. This procedure describes the process for preparing an in house positive urine ethanol control. The positive urine ethanol control is typically prepared in house.

B. Materials: 20 mL Class A Volumetric Flask
Analytical Balance (e.g., Tox Balance #3) or appropriate pipette

C. Reagents: Absolute Ethanol (100% Ethanol), ACS grade or better
Negative Urine

D. Prepare a positive urine ethanol control as follows:

1) Add approximately 10 mL of negative urine to a 20 mL Class A volumetric flask.

2) Place the 20 mL Class A volumetric flask on an analytical balance (e.g., Tox Balance #3) and tare the balance.

3) Drop by drop, add absolute ethanol to the 20 mL Class A volumetric flask. Listed below are a few examples relating target ethanol concentration and the target weight/volume of ethanol. Note that the amount of measured ethanol does not need to be exact.

Target Ethanol Concentration (g/100mL)	Target Ethanol Weight (g)	Target Ethanol Volume (µL)
0.060	0.0120	15.2
0.080	0.0160	20.3
0.100	0.0200	25.3

4) Record the weight or volume of absolute ethanol added to the 20 mL Class A volumetric flask on the Toxicology Reagent Preparation Log.

5) Add negative urine to the 20 mL Class A volumetric flask to the fiduciary mark to achieve a final volume of 20 mL. Mix the solution.

6) Calculate the concentration of ethanol using the following formula:

By Weight

$$\frac{\text{grams of ethanol}}{20\ \text{mL}} \times 100\ \text{mL} = \frac{\text{x grams ethanol}}{100\ \text{mL}}$$

Sample calculation when 0.0145 g of ethanol are measured:

(0.0145 g ethanol)/(20 mL) x (100 mL) = (0.073 g ethanol/100 mL)

By Volume

$$\frac{\mu\text{L of ethanol}}{20\ \text{mL}} \times \frac{0.789\ \text{g ethanol}}{\text{mL}} \times \frac{1\ \text{mL}}{1000\ \mu\text{L}} \times 100\ \text{mL} = \frac{\text{x grams ethanol}}{100\ \text{mL}}$$

7) Record the ethanol concentration to three decimal places. Standard rules of rounding apply.

8) Transfer the reagent to labeled containers (typically 2 mL per container). Store in a refrigerator or freezer. When stored in the freezer, the expiration date is one year from date of preparation or earliest expiration/use by/retest date of a component of the preparation, whichever is sooner. When thawed and stored in a refrigerator, this reagent has a 30 day expiration date from the date of thawing or the expiration date or frozen preparation, whichever is sooner.

9) Experimentally determine the ethanol concentration using Headspace GC-FID as outlined in section *5.6 Ethanol Analysis Quality Assurance*.

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5.6 Title: ETHANOL ANALYSIS QUALITY ASSURANCE

Purpose:

This section describes the procedures for performing quality control checks on reagents used for ethanol analysis. The following quality control checks are performed prior to a reagent being used for casework analysis.

Reagent:

Aqueous Ethanol Standards

Aqueous ethanol standards are used for preparing the calibration curve for ethanol analysis. These standards are purchased from a vendor (e.g., Cerilliant) at the four calibration levels (20 mg/dL, 80 mg/dL, 200 mg/dL, and 400 mg/dL).

1) Frequency: New lots of aqueous ethanol standards must be analyzed two times against a valid calibration prior to being used for casework analysis.

2) Interpretation: The mean of the experimentally observed concentration, expressed to the fourth decimal place, must be no greater than ± 5.0% of the manufacturer's certified value. For ethanol concentrations < 0.05 g/100mL, results must be no greater than ± 0.005 g/100mL. Standard rules of rounding apply.

3) Record your results on the Toxicology Material QC Log form. Store the form in the LIMS Resource Manager.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Aqueous Ethanol LOD Check

A positive aqueous ethanol standard is used to ensure that the GCHS instrument can detect ethanol at the administratively defined LOD. This standard is purchased from a vendor (e.g. Cerilliant) at 10 mg/dL.

1) Frequency: New lots of aqueous ethanol standard must be analyzed two times against a valid calibration prior to being used for casework analysis.

2) Interpretation: The observed concentration, expressed to the fourth decimal place, must be no greater than 0.02 g/100mL.

3) Record your results on the Toxicology Material QC Log form. Store the form in the LIMS Resource Manager.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Mixed Volatile Resolution Check

A mixed volatile resolution check consisting of five target components (acetaldehyde, acetone, methanol, ethanol and 2-propanol) is analyzed to demonstrate the resolution of these components. The mixed volatile resolution check is quality control checked as follows:

1) Frequency: Analyze each new lot number two times prior to being used for casework analysis.

2) Interpretation: The retention times obtained with the new standard must be no greater than ± 3% of the retention times that were obtained with the old standard. No extraneous peaks can be present.

3) Record your results on the Reagent Preparation Log by indicating QC method (GCHS), “passed” or “failed”, your initials, and date of QC check.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

1-Propanol Blank

A blank consisting of only the internal standard is analyzed to demonstrate no interference with ethanol. The 1-propanol blank is quality control checked as follows:

1) Frequency: Analyze each new lot number two times prior to being used for casework analysis.

2) Interpretation: Ensure one peak is present. It must be at the retention time for 1-propanol ± 3%.

3) Record your results on the Reagent Preparation Log by indicating QC method (GCHS), “passed” or “failed”, your initials, and date of QC check.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Negative Whole Blood Control

A negative whole blood control is typically purchased from a vendor (e.g., UTAK). Alternatively, an in house preparation of a negative whole blood ethanol control can be utilized.

1) Frequency: Analyze each new lot number two times and compare to a valid calibration prior to being used for casework analysis. Analyze once with internal standard and once without internal standard using 1000 µL of purified water to replace the internal standard volume.

2) Interpretation: Ethanol results for samples run with internal standard must be 0.000 g/dL. For samples run without internal standard, chromatograms should be free of ethanol and n-propanol peaks.

3) Record your results on the Toxicology Material QC Log form. Store the form in the LIMS Resource Manager.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Positive Whole Blood Control

A positive whole blood ethanol control is typically purchased from a vendor (e.g., UTAK). The concentration of ethanol in the positive whole blood ethanol control must be between 60 – 100 mg/dL. Alternatively, an in house preparation of the whole blood ethanol control can be utilized.

For commercial positive whole blood controls:

1) Frequency: The target concentration is determined for each new lot number of material by repeated analysis and calculation of the mean concentration using valid calibrations. A new lot must be analyzed 20 times over multiple days. The quality control check should be performed by different analysts on different instruments when practical.

2) Interpretation: The mean concentration of ethanol must not fall outside of the manufacturer's established range for a specific control lot number.

3) Record your results on the Positive Ethanol Control form. Indicate the QC method (GCHS), "passed" or "failed", and your initials. Store the Positive Ethanol Control form and the QC packets in the LIMS Resource Manager.

4) Establishing the positive whole blood control ethanol concentration: The whole blood control ethanol concentration is determined by truncating the mean ethanol concentration to the third decimal place. The acceptable range of values is ± 0.005 g/100mL of the established concentration.

5) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

For in house preparations of positive whole blood controls:

1) Frequency: The target concentration is determined for each new lot number of material by repeated analysis and calculation of the mean concentration using valid calibrations. A new lot must be analyzed 20 times over multiple days. Results shall be recorded on the Positive Ethanol Control form. The quality control check should be performed by different analysts on different instruments when practical.

2) Interpretation: The mean concentration of ethanol must be no greater than ± 10% of the calculated ethanol concentration.

3) Record your results on the Reagent Preparation Log with QC method (GCHS), your initials, and date of QC check.

4) Establishing the positive whole blood control ethanol concentration: The positive whole blood control ethanol concentration is determined by truncating the mean ethanol concentration to the third decimal place. The acceptable range of values is ± 0.005 g/100mL of the established concentration.

5) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Negative Urine Control

A negative urine control is typically prepared in house. Alternatively, a negative urine control can be purchased from a vendor.

1) Frequency: Analyze each new lot number two times against a valid calibration prior to being used for casework analysis. Analyze once with internal standard and once without internal standard using 1000 µL of purified water to replace the internal standard volume.

2) Interpretation: Ethanol results for samples run with internal standard must be 0.000 g/dL. For samples run without internal standard, chromatograms should be free of ethanol and n-propanol peaks.

3) Record your results on the Toxicology Material QC Log form. Store the form in the LIMS Resource Manager.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Positive Urine Control

A positive urine ethanol control is typically prepared in house. The concentration of ethanol in the positive urine ethanol control must be between 60 – 100 mg/dL. Alternatively, a positive urine control can be purchased from a vendor.

For in house preparations of positive urine controls:

1) Frequency: The target concentration is determined for each new lot number of material by repeated analysis and calculation of the mean concentration using valid calibrations. A new lot must be analyzed 20 times over multiple days. Results shall be recorded on the Positive Ethanol Control form. The quality control check should be performed by different analysts on different instruments when practical.

2) Interpretation: The mean concentration of ethanol must be no greater than ± 10% of the calculated ethanol concentration.

3) Record your results on the Reagent Preparation Log with QC method (GCHS), your initials, and date of QC check.

4) Establishing the positive urine control ethanol concentration: The positive urine control ethanol concentration is determined by truncating the mean ethanol concentration to the third decimal place. The acceptable range of values is ± 0.005 g/100mL of the established concentration.

5) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

For commercial positive urine controls:

1) Frequency: The target concentration is determined for each new lot number of material by repeated analysis and calculation of the mean concentration using valid calibrations. A new lot must be analyzed 20 times over multiple days. The quality control check should be performed by different analysts on different instruments when practical.

2) Interpretation: The mean concentration of ethanol must not fall outside of the manufacturer's established range for a specific control lot number.

3) Record your results on the Positive Ethanol Control form. Indicate the QC method (GCHS), "passed" or "failed", and your initials. Store the Positive Ethanol Control form and the QC packets in the LIMS Resource Manager.

4) Establishing the positive urine control ethanol concentration: The positive urine control ethanol concentration is determined by truncating the mean ethanol concentration to the third decimal place. The acceptable range of values is ± 0.005 g/100mL of the established concentration.

5) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

Positive Aqueous Controls

Positive aqueous ethanol controls are purchased from a vendor (e.g., Lipomed) and have ethanol concentrations between 0.02-0.40 g/dL. These controls have different ethanol concentrations that span the range of the standard curve (e.g., 0.020 g/dL, 0.150 g/dL, 0.400 g/dL). Note that the source of the positive aqueous controls must be different from that used for calibration of the standard curve.

1) Frequency: New lots of aqueous ethanol standards must be analyzed two times against a valid calibration prior to being used for casework analysis.

2) Interpretation: The mean of the experimentally observed concentration, expressed to the fourth decimal place, must be no greater than ± 5.0% of the manufacturer's certified value. For ethanol concentrations < 0.05 g/100mL, results must be no greater than ± 0.005 g/100mL). Standard rules of rounding apply.

3) Record your results on the Toxicology Material QC Log form. Store the form in the LIMS Resource Manager.

4) See Chapter 5.0 Ethanol Analysis by Dual Column GC Headspace for routine checks run concurrently with a blood alcohol batch.

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6.0 Title: QUALITY ASSURANCE

6.1 Contamination Prevention

Contamination prevention should include the following:

- Work surfaces must be kept clean.
- Reusable items (glassware, spatulas, etc.) must be cleaned before use.
- Disposable items are used only once. Disposable tips for repeater pipettes are the exception. These may be reused for acid solutions, base solutions, and buffer solutions. Replace tips if contamination is present.
- Aerosols
  - Dustoff will not be used and stored in the toxicology laboratory.
  - If a service engineer requires Dustoff, or other aerosols or volatile compounds, to isolate leaks in an instrument, the use of Dustoff will be communicated to the Toxicology Detail, and blood alcohol analysis will not be conducted. Moreover, the Dustoff will be removed from the laboratory directly after use.

6.2 Reagent Preparation

6.2.1 Reagent Prep Log

A Reagent Prep Log form in the Resource Manager in LIMS is used to document reagent preparation. The log will contain the following information:

- Identity of the reagent, including concentration, pH, molarity, etc., if applicable
- Internal lot number (the six numbers following the (T) or (Br) is the date the reagent was prepared). For example T031220-01.
- Initials and P# of person preparing reagent
- Expiration date of the reagent
- Ingredients and their lot numbers and expiration dates
- Item number of measuring device, if applicable
- Quality control checks performed and results
- Approval if necessary

6.2.2 Reagent Label

In addition, the container containing the reagent should bear the following information:

- Identity of the reagent
- Internal lot number
- Expiration date of reagent, if applicable
- Initials of the preparer
- Storage requirements

6.2.3 Pipettes for Methanolic Solutions

A positive-displacement pipette must be used to measure methanolic solutions for quantitative or semi-quantitative purposes.

6.3 Reference Materials

The laboratory maintains a collection of reference materials. Reference materials may be used as calibration standards in case work, for qualitative identification, for quality control purposes, and in the preparation of solutions of known concentration for use in quantitative methods.

When a reference material is used to establish a calibration curve or cutoff concentration, the reference material will be certified reference material traceable to the National Institute of Standards from an accredited reference material provider (e.g., ISO Guide 34) when available. Certified reference material may also be used to prepare controls.

Exception: Kit calibrators/controls for EMIT and ELISA may be obtained from the manufacturer.

Appropriate personal protective equipment (PPE) should be worn for safe handling of reference materials. Manufacturer's recommendations located in the Material Safety Data Sheets/Safety Data Sheets (MSDS/SDS) are to be followed regarding storage and transportation.

6.3.1 Quality Control Checks of Drug Stock and Working Solutions

Certified Reference Material is typically diluted to prepare stock and working solutions. A stock solution represents an intermediate solution obtained by diluting CRM; a stock solution is further diluted to prepare a working solution used for routine casework analysis. This section outlines the quality control measures required for drug stock and working solutions prior to using them in casework analysis.

6.3.1.1 Drug Stock Solutions

Stock solutions do not require a quality control check via instrumental analysis. However, the preparation of stock solutions will be reviewed by a Toxicology Manager, Toxicology Supervisor or a second Forensic Scientist authorized to perform work in that discipline. The reviewer will verify that the stock solution was prepared according to the requirements of the Toxicology Technical Manual, that lot numbers and expiration dates of all ingredients written on the Reagent Prep form are correct, and that the storage container has been correctly labeled. After verifying all information, the reviewer will place their initials, P# and the date on the "Approved By/Date:" section of the Reagent Prep form. The reviewer will also initial the drug stock solution storage container to indicate that the reagent has been approved for use.

6.3.1.2 Drug Working Solutions

Newly prepared lots of working solutions will be verified using the applicable instrument method (e.g., ELISA, EMIT, GC/MS or LC/MS/MS) prior to being used for casework analysis. A typical quality control check is performed by comparing a current lot of working solution to a newly prepared lot of working solution from a different source (e.g., a current standard working solutions is used to verify a new lot of a control working solution). Note that a newly prepared standard working solution may be verified by comparison with a newly prepared control working solution. If both the standard and the control working solutions are being quality control checked simultaneously, it is best practice to have different analysts prepare each solution.

Immunoassay working solutions are quality control checked by using the entire drug screen methodology. The instrument is calibrated with a standard working solution as outlined in the method, followed by analyzing the positive control in duplicate. QC samples should be placed directly following the first positive control. All QC criteria stated in the Batch Acceptance Criteria must be met. In addition, optical density/absorbance values should be comparable to previous calibrator/standard or control results.

Confirmation working solutions are quality control checked by preparing unextracted specimens or by using the entire extraction methodology. The instrument is calibrated with a standard working solution as outlined in the method, followed by a negative control. The control working solution is checked at concentrations equal to each calibration standard used in the method. All QC criteria stated in Chapter 4.0 Confirmation Testing must be met on the batch.

Newly prepared lots of internal standard working solutions are quality control checked using the applicable Confirmation method in duplicate. Internal standard working solutions are quality control checked by preparing unextracted specimens or by using the entire extraction methodology. Resulting chromatograms should be free of any additional/interfering chromatographic peaks. Retention time and ion ratios (if applicable) shall pass criteria stated in chapter 4.0 Confirmation Testing.

The results of a QC check are stored in the LIMS and are documented on the “QC Method:”, “QC Result:” and “QC By/Date:” sections on the Reagent Prep log form. Both the preparation and the quality control check of a working solution will be reviewed by a Toxicology Manager, Toxicology Supervisor or a second Forensic Scientist authorized to perform work in that discipline. The reviewer will verify that the solution was prepared according to the requirements of the

Toxicology Technical Manual, that lot numbers and expiration dates of all ingredients written on the Reagent Prep form are correct, and that the storage container has been correctly labeled. After verifying all information, the reviewer will place their initials, P# and the date on the "Approved By/Date:" section of the Reagent Prep form. The reviewer will also initial the drug stock solution storage container to indicate that the reagent has been approved for use.

6.4 Controls

Positive controls are included in casework batches to monitor the calibration of each batch. Positive control data from casework batches and from other batches that undergo the entire specimen processing methodology (e.g., extracted QC checks) are logged into spreadsheets and reviewed for trends by the analyst entering the data. Levy-Jennings charts are automatically updated for blood alcohol and drug confirmation QC. The analysts will indicate that they have checked the data for trends by entering their initials onto the spreadsheet. Statistical techniques are also applied to the blood alcohol and drug confirmation data when measurement uncertainty is updated.

Spreadsheets are located at:

H:\CB\Forensics\Toxicology\Tox- QC\Blood Screens
H:\CB\Forensics\Toxicology\Tox- QC\Blood Ethanol Control Data
H:\CB\Forensics\Toxicology\Tox- QC\Drug Confirmation Control Data

Negative controls are included in casework batches to verify the absence of interfering substances or contamination in the reagents and materials used for that method, and to test for carryover on each batch.

6.5 Negative Matrix

6.5.1 Negative Blood

Negative whole blood may be purchased from an outside vendor or supplied in-house. Negative whole blood purchased from vendor will be stored according to the vendor's storage requirement. Negative whole blood supplied in-house may be stored refrigerated in the collection tubes or frozen if transferred to different storage containers.

6.5.1.1 Quality Control

ELISA - Analyze in duplicate against the current lot of working stock solution on a screening batch. QC samples should be placed directly following the first positive control. All QC criteria stated in the Batch Acceptance Criteria section in Chapter 3.0 must be met on each batch. Negative whole blood results must be negative for all drug classes.

Drug Confirmation – Analyze each new lot number two times and compare to a valid calibration prior to being used for casework

analysis. Analyze once with internal standard and once without internal standard. See Section 4.0.4.3 - Negative Control for acceptance criteria.

Additionally, new lots of negative whole blood shall be checked for ionization suppression/enhancement. For each LCMSMS method, extract a set of standards in both the old lot of blood and the new lot of blood. The extracted batch should contain a negative, low, and high control extracted in the old lot of blood. Results of the new lot should pass quantitative, Target:ISTD response ratio, and transition ratio QC.

Results should be recorded on the Toxicology Material QC Log form and stored in LIMS.

Blood Ethanol – See Chapter 5.6 Ethanol Analysis Quality Assurance for QC requirements.

Documentation – Record results on the Toxicology Material QC Log.

6.5.2 Negative Urine

Negative Urine may be purchased from an outside vendor or supplied in-house. It will be stored frozen, then refrigerated once thawed.

6.5.2.1 Quality Control

See Chapter 5.6 Ethanol Analysis Quality Assurance for urine ethanol QC requirements.

Documentation – Record results on the Toxicology Material QC Log.

6.6 Critical Supplies

All primary reference materials are considered critical supplies. A primary reference material vendor list is available electronically in Qualtrax. Approved vendors for purchasing of critical supplies will be reviewed and should meet the following criteria:

- If available, suppliers of certified reference material used to establish or maintain measurement traceability shall be either:
  a) a National Metrology Institute that is a signatory to the BIPM1 - CIPM Mutual Recognition Arrangement with the certified reference material listed in the BIPM key comparison database (KCDB)2, or
  b) an accredited reference material producer that is accredited to ISO 17034:2016 by an accrediting body that is a signatory to a mutual or multilateral recognition arrangement in an ILAC recognized regional accreditation cooperation or the ILAC Mutual Recognition Arrangement, with a scope of accreditation covering the certified reference material.

In situations where a reference material producer that meets ISO 5.6.3.2.1 is not available, the laboratory must confirm competence, measurement capability and measurement traceability for the supplier and product being purchased. Objective evidence of the confirmation shall be available for review. In these situations, Toxicology Manager will evaluate the ability to continue using the vendor and issue a signed memo attesting to the appropriateness of the vendor.

A record of the evaluation and/or a copy of the vendor's ISO accreditation certificate will be maintained with the vendor list, referenced above.

6.7 Chemical and Drug Inventory

Certified reference materials of quantitative solutions of drugs and metabolites are available from various vendors. Any quantitative solution that is shipped with a certificate of analysis may be used for the same purposes as the above mentioned reference material. As such CRMs are received into the laboratory; its corresponding certificate of analysis is placed on file. Quantitative certificates of analysis are kept on file for a minimum of 5 years. The manufacturer's stated prepared concentration should be the value used if the certificate states both a prepared concentration and an analyzed concentration.

6.8 Expiration Dates

The expiration date of a chemical/reagent/reference material/native matrix is defined as the manufacturer's expiration/use by/retest/best before/minimum shelf life date, with exceptions noted below, or elsewhere in this manual. Chemicals/reagents/reference materials/negative matrices will not be used beyond the expiration date. If a chemical/reagent/reference material/negative matrix will expire before an analysis is complete, it will not be used for the analysis. If a manufacturer updates the retest date, the updated retest date may be used when preparing new reagents if the manufacturer's certificate of analysis on file is updated; expiration dates of previously prepared reagents will not be changed. Additionally, a stability study may be conducted at the Laboratory in order to extend expiration dates.

6.8.1 Chemicals

If a chemical is received into the lab without an expiration date, the expiration date will be researched online (e.g., Certificate of Analysis) or by contacting the manufacturer directly. This applies when using chemicals from other Details as well. If research yields no expiration date, an expiration date of five years from the date of receipt will be applied.

6.8.2 Reference Material

Reference material without an expiration will receive an expiration date of one year from date of receipt. Reference material will be stored as outlined by the manufacturer.

6.8.3 Opening Reference Material

When an ampule of reference material is opened and not entirely consumed, the remaining reference material may be transferred to a vial and capped. The open date will be noted on the vial and the expiration date will be set to the manufacturer's expiration date or 1 year from the date of opening, whichever occurs first. The vial will be stored as outlined by the manufacturer.

6.8.4 Dilution of Reference Material

When reference material is diluted to prepare a stock or working solution, the expiration date of the newly prepared solution will be set as that of the earliest expiring reagent or 1 year from the date of preparation, whichever occurs first. Dilutions of reference material will be stored as outlined in Section 4.2.

6.8.5 Distilled Water

The expiration date of distilled water from Sparkletts, or similar vendor, will be "Until Consumed."

Note: Sections 6.9.3 and 6.9.4 represent new criteria which apply to preparations made on or after the approval date of the May 2017 Toxicology Technical Manual revision. Expiration dates of existing preparations will remain as listed on the reagent preparation logs.

6.8.6 In-house Negative Matrix

The expiration date of negative blood and urine supplied in-house will be set to 1 year from date of collection. The expiration date may be extended if further testing is performed to evaluate the integrity of the lot number. Once thawed, the expiration date for in-house blood and urine is 30 days for all analyses except Cannabinoids in Blood.

6.8.7 Negative Matrix for Cannabinoids in Blood

Once thawed, the expiration date for negative matrix for Cannabinoids in Blood is seven (7) days.

6.9 Instrumentation

An instrument log book, located near each instrument, is maintained to document all repairs, maintenance, and record tunes (if applicable) performed on the instrument.

Maintenance on instrumentation may be performed as a result of routine preventative maintenance performed by the manufacturer, their contractors, or laboratory staff. In addition, any of these parties may undertake maintenance or troubleshooting to address problems or malfunctions. Documentation of any maintenance, repairs, or problems shall be recorded in the instrument maintenance logbooks.

6.9.1 GC/MS

GC/MS instrumentation is tuned prior to use on a daily basis when employed in electron impact ionization mode. When a large GC/MS sample batch continues over multiple days, the run sequence does not need to be

interrupted to tune the instrument. It is not necessary to tune the instrument on days it is not being used. Tuning data will be maintained for one year at the instrument site.

6.9.2 LC/MS/MS

An autotune or checktune is performed each day the LC/MS/MS is used prior to analysis. When a large sample batch continues over multiple days, the run sequence does not need to be interrupted to tune the instrument. It is not necessary to tune the instrument on days it is not being used. Tuning data will be maintained for one year at the instrument site.

6.9.3 Immunoassay / ELISA (Blood)

Prior to analyses, a valid Self Test is performed on the screening instrument. A valid Self Test is one in which all tests pass.

6.9.4 The Artel Pipette Tracker System

The Artel Pipette Tracker System cannot operate outside of a certain temperature window per the manufacturer. No maintenance log entry is necessary because the instrument self-checks and will not allow testing to continue if temperature is not within the adequate range.

6.10 Measuring Equipment

6.10.1 Pipettes

Calibration of pipettes used for pipetting calibration standards, controls, internal standards, negative matrix, and casework samples, as well as for preparing calibration standard, control, and internal standard stock and working solutions will be checked using the Artel Pipette Tracker system on a quarterly basis, after being dropped, or if other damage is suspected. Adjustable pipettes will be checked at volumes to encompass the minimum and maximum volumes used.

Each volume will be checked four times. If extenuating circumstances cause a volume to be pipetted incorrectly, a fifth check may be performed and the incorrect volume may be discarded from the run. Data from both runs will be saved and the reason for the dropped check will be documented. If the pipette does not meet the criteria specified in Chapter 8.0- Quality Control Plan, the corrective action steps detailed in the Quality Control Plan will be followed. Refer to the Artel Manufacturer's User Manual/Guide for instructions on usage.

Pipette checks may also be completed by weighing distilled water on the TOX #3 Mettler balance (record on Toxicology Pipette Performance Check Record Form).

Results of the pipette check will be verified by the Toxicology Manager/Toxicology Supervisor/designee. Results will be stored in Resource Manager.

Note: MLA pipettes are used exclusively as transfer pipettes and are not used for the metrological preparation of calibrators, controls, negative matrix or casework samples. Therefore, MLA pipettes are not used to establish measurement traceability and do not require external annual calibration or internal performance checks.

6.10.2 Serialized Glassware

If glassware is used when diluting certified reference material to prepare calibration standard stock and working solutions, it will be serialized glassware. Serialized glassware may be used to prepare controls and internal standards.

6.10.2.1 Calibration

Serialized glassware will be calibrated by an accredited calibration service supplier prior to use. Recalibration shall recur at least once every ten years by an appropriately accredited calibration service supplier.

6.11 Safe handling, use, transportation and storage of measuring equipment

Manufacturer's Operating or Instruction Manuals (see Chapter 9.0 References for further details) should be referred to when there are concerns about the handling, usage, and storage of the following measuring equipment. Measuring equipment is not transported outside the Forensic Laboratory, except for repairs. Contact the manufacturer for transportation instructions.

- Balances
- Pipettes
- Diluter/dispenser
- GCHS
- GC/MS
- LC/MS/MS
- Artel Pipette Tracker system

6.11.1 Thermometers

Thermometers will be handled with appropriate personal protective equipment. When not in use, it is best to store thermometers in an upright position or at an angle of 15˚ or more. Use a special tray or rack to store thermometers properly. If deemed unsuitable for use, it will be disposed of in the appropriate receptacle. Thermometers are not transported outside of the Forensic Laboratory.

6.11.2 Glassware

Glassware used for measuring will be handled with appropriate personal protective equipment. It will be clean and inspected prior to use to be free of cracks and/or chips. If glassware is deemed unsuitable for use, it will be disposed of in an appropriate glass receptacle. Glassware is stored in the

Toxicology Lab. Serialized glassware is stored in a different location than non-serialized glassware. Glassware is not transported out of the Forensic Laboratory.

6.12.2.1 Glassware used for Reagent Preparation

The preparer will rinse the glassware with methanol directly following the preparation, prior to delivering the glassware to the wash station.

6.12 Immunoassay Drug Screening

Known Possible Sources of Error:

- Cross-reactivity of structurally related compounds at certain concentrations will produce false positive qualitative results.
- Use of kits and their components at, near or beyond the stated expiration dates.
- Not allowing substrates, conjugates, standards and controls to come to room temperature prior to use.
- Bubbles present in samples.
- Use of pipettes which are not in working order.
- Sodium azide, a common antimicrobial agent, will block the activity of the enzyme horseradish peroxidase (for ELISA assays).
- Interchanging plates and conjugate with different lot numbers. Kit plates and conjugate are validated based on component kit lot numbers (for ELISA assays).
- Not performing a “Start of Day Wash” prior to analysis (for ELISA assays).
- Substrate reagent which has developed an obvious color change.
- There is possibility that substances and/or factors not listed (e.g., technical or procedural errors) may interfere with the test and cause false results.

6.13 Refresher Training

Qualified Forensic Scientists/Toxicology Supervisor who have been on leave for 90 days or more must undergo a brief refresher training prior to resuming independent casework or case review. At the minimum, refresher training will consist of the following:

- Receive an update from the Toxicology Manager/Supervisor on new policies and the current status of the Detail
- Review chapters in the Toxicology Technical Manual that pertain to their current assignment
- Read old emails
- Review Department General Orders (GO’s) and Procedural Orders (PO’s) on UMLV, if applicable

If the leave was for 180 days or more the following will also be completed:

- Complete one supervised batch of casework samples in the area of their current assignment

The Toxicology Manager/Supervisor will document the tasks of the Refresher Training on LVMPD 311 Statement of Performance Cover Page Form, and will discuss the plan with the Forensic Scientist/Toxicology Supervisor upon their return to duty.

6.14 Back-up of Electronic Records

Instrumental data are collected and/or stored in designated folders on the instrument computer. Data in the designated folders are backed up to a remote server by LVMPD's Information Technologies Bureau (ITB). Data on the remote server are maintained indefinitely by ITB.

6.15 Method Validations

Each method listed in this manual was subjected to a validation procedure. The results of the method validations are kept in Qualtrax.

Modifications to a validated method require evaluation to confirm that the changes do not have an adverse effect on the method's performance. The decision regarding which performance characteristics require additional validation is based on consideration of the specific parameters likely to be affected by the change(s). These changes may include, but are not limited to:

- Analytical conditions
- Instrumentation
- Sample processing
- Data software

For example, changes of extraction solvent or buffer may affect linearity, interferences, LLOQ, precision, and bias. A change of the analytical column stationary phase or a change in mobile phase composition may affect linearity and interferences. Further, consideration should be given to conducting parallel studies with known or proficiency samples utilizing both a previously validated method and the modified method to evaluate the effects of the changes. The goal is to demonstrate the impact the changes have on the performance of the previously validated procedure. (*Copyright holder and publisher- AAFS Standards Board)*

6.16 Proficiency Tests

6.16.1 Use of Internally Created Proficiency Tests

Internally created proficiency tests can consist of the following:

- Negative matrix (blood and/or urine) and negative matrix spiked with a known concentration of one or more analytes
- Previously analyzed casework samples
- A combination of spiked samples, negative matrix, and previously analyzed casework samples

Internally created proficiency test samples will first be analyzed by a different

forensic scientist who is authorized to perform the analysis.

6.16.2 Passing Criteria for Proficiency Tests

6.16.2.1 External Tests

Vendor - College of American Pathologist (CAP)
Tests - Alcohol (blood) and drugs (blood)
Passing Criteria - Acceptable grade from CAP

Vendor - Collaborative Testing Services, Inc. (CTS)
Test – Alcohol (blood)
Passing Criteria - ±25% or ± 2 SD of the grand mean

6.16.2.2 Internally Created Tests

Blood Alcohol - ±10% of initial result

Drug Screens - Results should agree with initial results, that is, samples that were previously positive or negative should result in the same. Results that were previously close to the cutoff may have the opposite result.

Drug Confirmations - ±30% of initial results

6.17 Technical and Administrative Review of QC Packets

A Technical and Administrative Review will be performed on all QC Packets prior to the review of cases. The technical reviewer will complete the Technical and Administrative Review form attached to the QC Packet.

6.18 Technical and Administrative Review of Cases

Technical and Administrative Reviews are to be conducted on all cases.

It is not necessary to access documents outside of the Lab Case/Unit Record Details in LIMS, the Quality Control Packets in Qualtrax, Measurement Uncertainty Summary, the Toxicology Technical Manual, and the Forensic Laboratory Quality Manual on a routine basis when performing technical and administrative reviews.

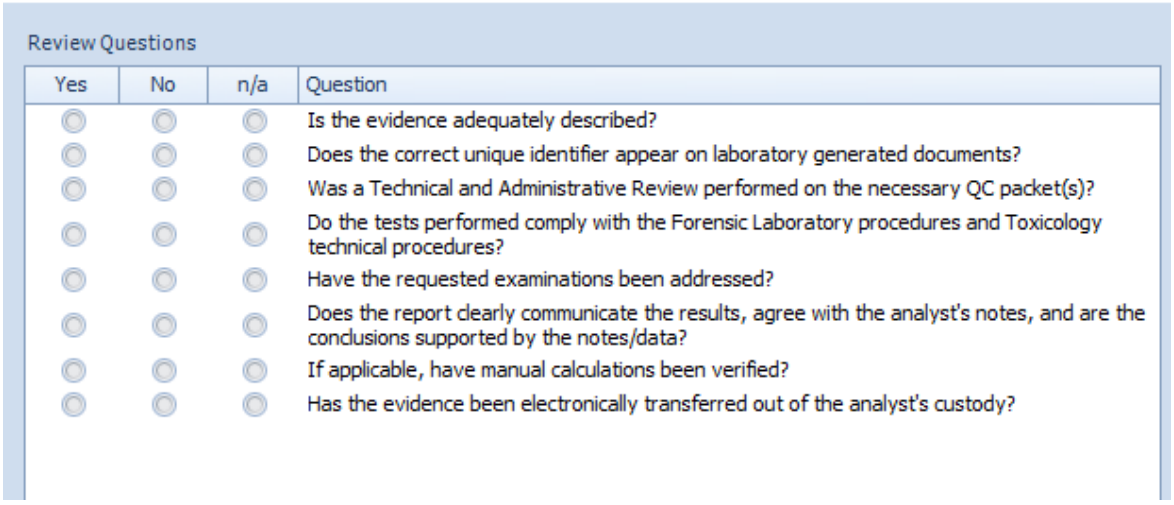
6.18.1 Technical Review

Refer to the Forensic Laboratory Quality Manual section 7.7.1 l) Technical Review of Technical Records and section 7.5 Technical Records. The technical reviewer will verify that:

- The evidence is adequately described
- The correct unique identifier appears on laboratory generated documents
- A Technical and Administrative Review was performed on the necessary QC packet(s)

- The tests performed comply with the Forensic Laboratory Quality Manual procedures and Toxicology technical procedures
- The requested examinations have been addressed
- The report clearly communicates the results and agrees with the analyst's notes
- Manual calculations are correct, if applicable
- The evidence has been electronically transferred out of the analyst's custody

When the technical review is completed in LIMS, the reviewer will complete the technical review questions in LIMS. If YES or n/a criteria are not met, the case will be returned to the analyst with feedback regarding any necessary corrections.


Review Questions

Yes	No	n/a	Question
○	○	○	Is the evidence adequately described?
○	○	○	Does the correct unique identifier appear on laboratory generated documents?
○	○	○	Was a Technical and Administrative Review performed on the necessary QC packet(s)?
○	○	○	Do the tests performed comply with the Forensic Laboratory procedures and Toxicology technical procedures?
○	○	○	Have the requested examinations been addressed?
○	○	○	Does the report clearly communicate the results, agree with the analyst's notes, and are the conclusions supported by the notes/data?
○	○	○	If applicable, have manual calculations been verified?
○	○	○	Has the evidence been electronically transferred out of the analyst's custody?

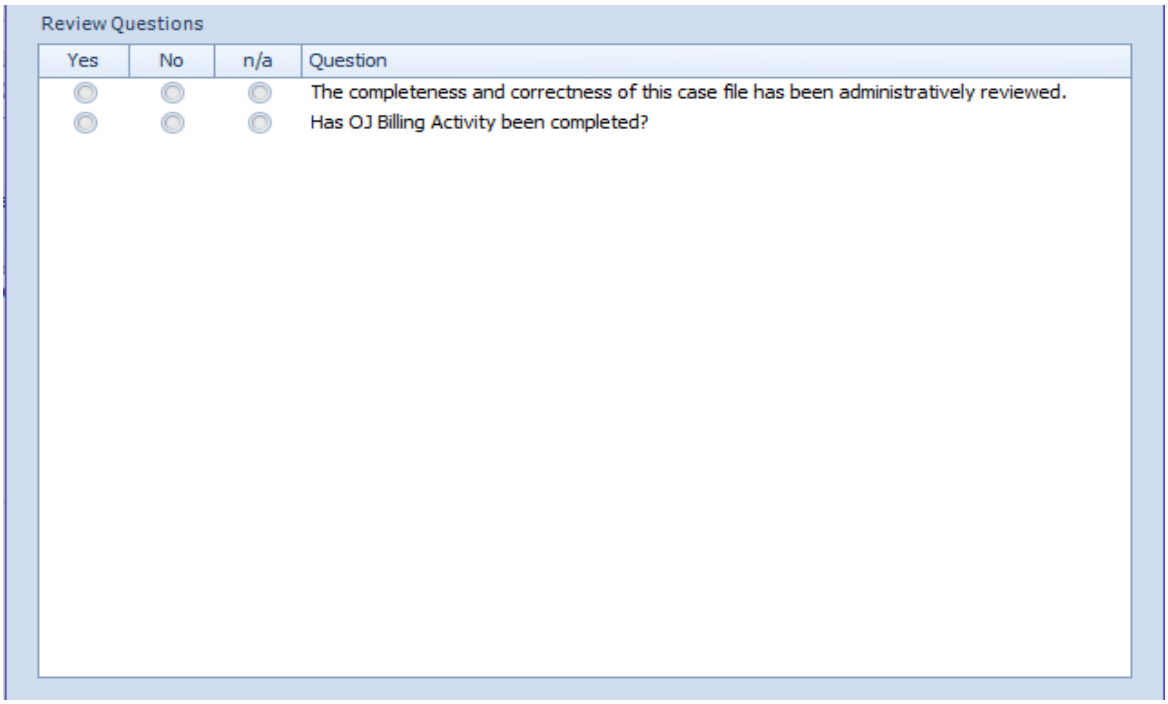
If the report is canceled in LIMS because the case was returned to the analyst during an administrative review, LIMS automatically assigns a new technical review. The technical review must be completed unless the administrative review was returned for any of the following reasons:

- Spelling and grammar that does not affect the results, opinion, or interpretations
- Minor punctuation edits that do not alter the meaning of the sentence of phrase

If any of the above criteria applies, the additional technical review can be withdrawn in LIMS.

6.18.2 Administrative Review

Refer to the Forensic Laboratory Quality Manual section 7.7.1.1 Administrative Review of Technical Records. If the case is complete and correct, document the administrative review in LIMS. In addition, verify that OJ billing activity has been completed if applicable.


Review Questions
Yes
No
n/a
Question
The completeness and correctness of this case file has been administratively reviewed.
Has OJ Billing Activity been completed?

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
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7.0 Title: PERFORMANCE CHECK OF THE HAMILTON DILUTER/DISPENSER

Procedure

A. Purpose and Principle: The purpose of this procedure is to verify the accuracy of the dilution volume and sample volume of the Hamilton Diluter/Dispenser. A gravimetric calibration method is used. It exploits the scientific principle that one milliliter of water weighs one gram at room temperature.
NOTE: 18 gauge tubing is used on the diluter/dispenser.

B. Frequency: Perform verification monthly and after replacing any parts.

C. Use a calibrated, analytical balance capable of weighing to 0.0001 g.

D. Record on Hamilton Diluter/Dispenser Performance Check Record form. Store in Resource Manager.

E. Measure the diluent volume.

1. Adjust the settings for the diluent syringe to a volume appropriate for the intended application, for example:
SYRINGE: 1000 µL VOLUME: 1000 µL

2. Adjust the settings for the sample syringe to either:
SYRINGE: 100 µL VOLUME: 0 µL

3. Use distilled water at room temperature. Prime the diluter/dispenser about 5 times and discharge the water to waste.

4. Position a clean, dry weigh boat, or equivalent receptacle on the balance. Tare the balance.

5. Load the syringe with distilled, room-temperature water. Discharge the water into the weigh boat or equivalent receptacle. Wait for the balance to stabilize and record the weight. Tare the balance. Repeat step 5 until you have obtained 10 results.

F. Measure the sample volume

1. Adjust the settings for the diluent syringe to:
SYRINGE: 1000 µL VOLUME: 0 µL

2. Adjust the settings for the sample syringe to a volume appropriate for the intended application, for example:
   SYRINGE: 100 µL VOLUME: 100 µL

3. Follow steps 4 and 5 above.

G. Calculate the mean for the diluent syringe. Do likewise for the sample syringe. Standard rules of rounding apply.

Interpretation

Individual weights of all syringes must fall within ± 3% of the target volume. For example:

A. Diluent Syringe (1000 µL): All weights must be 0.9700 g - 1.0300 g.
B. Sample Syringe (100 µL): All weights must be 0.0970 g - 0.1030 g.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

8.0 Title: QUALITY CONTROL PLAN

	Instrument	Frequency	Criteria	Corrective Action
Refrigerators/Freezers	Tox #1 Continental Refrigerator Model: 2R-SGD SN: A96E5471	**Internal:** Check temperature once every two weeks **External:** N/A	**Fridge:** 2 - 8 C **Freezer:** ≤ -15 C Use "Refrigerator/Freezer Temperature Log" Form found in Qualtrax. Forms will be completed electronically in the Resource Manager.	If a refrigerator/freezer does not meet criteria: 1. Check again within 2 hours. 2. Check thermometer against a second NIST thermometer. Replace if needed, then go directly to step 4. If thermometer is accurate, proceed through remaining steps. 3. Adjust thermostat. Note the adjustment that was done. 4. Monitor the temperature within 24 and 48 hours to ensure stability. 5. If the above steps do not correct the problem, tag out of use and advise lab manager or supervisor (prepare a Corrective Action Report, if needed). **NOTE:** If temperature deviates more than 3 degrees outside of the acceptable range after completing step 3, move contents to an operable unit. If refrigerator/freezer appears to be malfunctioning, immediately move contents to an operable unit.
	Tox #4 Frigidaire Refrigerator Model: FRU17B2JW9 SN: WA63001408			
	Tox #6 Sanyo Refrigerator Model: SRR-49GD-MED SN: KJ00000377M			
	Tox #7 Frigidaire Freezer Model: FFU21F5HWF SN: WB92448488			
	Tox #9 Isotemp Plus Refrigerator Fisher Scientific Model: MR72SS-GAEE-FS SN: 0142034601150928			
	Tox #10 Whirlpool Refrigerator/Freezer Model: WRT106TFDW01 SN: VS64638614			
	Tox #11 True Refrigerator Model: GDM-49-SCI-HC-TSL01 SN: 9255406			

	Instrument	Frequency	Criteria	Corrective Action
	Tox #12 True Refrigerator Model: GDM-49-SCI-HC-TSL01 SN: 9558563			
Serialized Glassware	1 mL. 5mL, 10 mL Class A Serialized Volumetric Flasks Calibration plan is located in Qualtrax.	**External:** Once every 10 years. **<u>Critical Service</u>** Vendor: Rice Lake / Heusser Neweigh, LLC. 1-925-798-8900	Volume must fall within calibration lab's tolerance.	None. If glassware does not meet calibration criteria it will be taken out of service.

	Instrument	Frequency	Criteria	Corrective Action
Pipettes	Pipette identifications and certification schedules are listed in Resource Manager. Calibration plan is located in Qualtrax.	**External:** Calibrate annually **<u>Critical Service</u>** Vendor options: Calibrate, Inc. 1-800-833-0511 Integrated Service Solutions, Inc. 1-610-287-3433 Quality Control Services, Inc. 1-800-843-1237 Rice Lake / Heusser Neweigh, LLC. 1-925-798-8900 **Internal:** Pipettes used to pipette standards, controls, internal standards, negative matrix, and casework samples, and for preparing standard, control, and internal standard stock and working solutions will be checked quarterly. Note: MLA pipettes are used exclusively as transfer pipettes and are not used for the metrological preparation of calibrators, controls, negative matrix or casework samples. Therefore, MLA pipettes are not used to establish measurement traceability and do not require external annual calibration or internal performance checks. After a send-out repair perform a routine check as described in Chapter 6.0 Quality Assurance.	**External:** See the Tox Diluter-Pipette Calibration Reference located at H:\CB\Forensics\Toxicology\Pipettes\Diluter-Pipette Calibration Reference for volumes to be calibrated and pass/fail accuracy percentages. Vendor certifications are kept in Resource Manager. **Internal:** 2 µL ≤ volume < 15 µL ±5% (actual or relative inaccuracy). CV% (imprecision) should not be greater than 3.000 on the Artel® Pipette Tracker™. Volume ≥ 15 µL ± 3% (actual inaccuracy). CV% (imprecision) should not be greater than 3.000 on the Artel® Pipette Tracker™. Performance checks will be completed using the Artel® Pipette Tracker™ system, or by weighing distilled water on Tox#3 Mettler balance. Completed checks will be stored in Resource Manager.	If a pipette is not operating properly: 1. Repeat the test. 2. Troubleshoot per manufacturer's recommendations. 3. If the pipette is still not operating properly, tag out of service. 4. Advise lab manager or supervisor who will arrange for repair, if necessary. 5. Prepare a Corrective Action Report, if necessary.

	Instrument	Frequency	Criteria	Corrective Action
Balances	Tox #3 Mettler Model: XS105DU SN: 1127113910 Tox #5 Ohaus Model: SPX222 SN: B618431040 Calibration plan is located in Qualtrax.	**External:** Calibrate annually **<u>Critical Service</u>** Vendor options: Mettler Toledo, Inc. (800) 523-5123 Precise Weighing 1-661-250-9044 Rice Lake / Heusser Neweigh, LLC 1-925-798-8900 **Internal:** Monthly performance checks, on balances that have a direct bearing on the severity of sentence (TOX # 3) – performed with ASTM 1 weight sets. After send-out repair, perform a monthly performance check.	**External:** See the Toxicology Balances Calibration Information located at H:\CB\Forensics\Toxicology\Balances for the minimum required levels of calibration and pass/fail accuracy information. **Internal:** Tox #3: ± 0.0002g for masses ≤50g ± 0.0003g for masses >50g Tox #5 ±0.03g for masses ≤100.00g ±0.1g for masses >100.00g Logbooks are located in Resource Manager.	If a balance is not operating properly: 1. Initiate manufacturer's procedures to perform a mechanical internal calibration (if applicable) or external calibration. 2. If the above steps do not correct the problem, tag out of use, advise the lab manager or supervisor (prepare a Corrective Action Report, if needed).
Fume Hoods	Tox #3 LABCONCO Model: 9840601 SN: 050739541B Tox #4 LABCONCO Model: 9840601 SN: 050739542B Tox #5 LABCONCO Model: 7280400 SN: 050639179H	**External:** Annually **Internal:** N/A For annual certification: Vendor Options: Controlled Environment Management (480) 836-4144 For repairs and maintenance: Vendor options: Thomas and Mack 896-7035	**External:** Meet external vendor criteria. Vendor certifications are located in Resource Manager.	If a fume hood is not operating properly: 1. Tag out of use. 2. Advise lab manger or supervisor.

	Instrument	Frequency	Criteria	Corrective Action
	Tox #6 LABCONCO Model: 7280400 SN: 050639181H			
Critical Thermometers	Thermometer identifications and certification schedules are listed in Resource Manager. See Refrigerator/Freezer Temperature Log for location information.	**External:** N/A NIST Thermometers – Replace every two years or sooner per manufacturer's guidelines.	N/A	N/A

	Instrument	Frequency	Criteria	Corrective Action
Non-Critical Thermometers	Thermometer identifications, certification schedules, and locations are listed in Resource Manager.	**External:** N/A NIST Thermometers – Replace every two years or sooner per manufacturer's guidelines	N/A	N/A
Diluter/Dispensers	Tox #6 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600BD1621 Controller SN: MD600BC1527 Tox #7 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600GH10521 Controller SN: ML600GG10491 Tox #8 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600GJ10733 Controller SN: ML600GH10667	**External:** Calibrated annually **<u>Critical Service</u>** Vendor options: Calibrate, Inc. 1-800-833-0511 Integrated Service Solutions, Inc. 1-610-287-3433 Quality Control Services, Inc. 1-800-843-1237 Rice Lake / Heusser Neweigh, LLC. 1-925-798-8900 **Internal:** When in use: Conduct a performance check of the syringe monthly, replace as needed. Verification must be done every time any parts, other than tubing, are replaced. After send-out repair: Conduct a performance	**External:** See the Tox Diluter-Pipette Calibration Reference located at H:\CB\Forensics\Toxicology\Pipettes\Diluter-Pipette Calibration Reference for volumes to be calibrated and pass/fail accuracy percentages. Vendor certifications are located in Qualtrax. **Internal:** Diluent and Sample syringes: weight =± 3% of volume checked Use "Hamilton Diluter/Dispenser Performance Check Record" Form located in Qualtrax. Logbooks and Verifications are located in Resource Manager.	If the diluter/dispenser does not meet criteria: 1. Repeat test. 2. Troubleshoot per manufacturer's recommendations. 3. Verify balance accuracy. 4. If the above steps do not correct the problem, tag out of use, advise lab manager or supervisor and prepare a Corrective Action Report, if needed.

	Instrument	Frequency	Criteria	Corrective Action
	Tox #9 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600JB12659 Controller SN: ML600JB12678	check. Hamilton (800) 648-5950 Diluter/Dispensers not used routinely (stored and used as back-ups) shall have a performance check conducted prior to use and monthly thereafter if kept in use.		
Ovens	Tox #1 Oven (Gravity Oven) VWR Model: 1330 GM SN:1000599	**External:** N/A **Internal:** Temperature is checked against a NIST thermometer before use.	N/A	If oven does not meet criteria: Adjust temperature setting until thermometer displays temperature appropriate for procedure. If the oven cannot maintain the appropriate temperature. tag out of use, advise lab manager or supervisor and prepare a Corrective Action Report, if needed.

	Instrument	Frequency	Criteria	Corrective Action
GC/MS	Tox #9 GCMS GC Model: Agilent 7890A SN: CN10521043 MS Model: Agilent 5975C SN: US10523720 Tox #10 GCMS GC Model: Agilent 7890A SN: CN10501124 MS Model: Agilent 5975C SN: US10494606	**External:** Refer to specific instrument contract/agreement for appropriate support phone numbers and service agreement **Internal:** Before use (once/day) and after send-out repair.	**External**: Meet external vendor criteria. **Internal:** **MS performance -** Autotune must be performed each day that the instrument will be used, prior to analysis. N_2 should not be greater than 10% for EI mode. **Maintenance (monthly):** Check rough pump oil. **Maintenance(as needed):** Change septum & liner, clean source, change gold seal, trim/replace column, change syringe. Logbooks are located in lab area near equipment. Documentation may be archived in Resource Manager.	At a minimum, attempt the following corrective action if any of the performance checks fail: 1. Repeat test. 2. Troubleshoot using manufacturer's recommendations as outlined in the Chemstation Users Guide. 3. Call for technical support. 4. Tag out of use. 5. Advise lab manager or supervisor and call for a service engineer. 6. Record problem in the instrument maintenance manual or, if necessary, on a Corrective Action Report.
LC/MS/MS	Tox #1 LCMSMS LC Model: Agilent 1260 Infinity Series SN: See instrument maintenance manual for separate components MS Model: Agilent 6420 MS/MS SN: SG15277008	**External:** Refer to specific contract/agreement for appropriate support phone numbers and service agreement **Internal:** Before use (once/day) and after send-out repair.	**External:** Meet external vendor criteria. **Internal:** **MS performance –** Checktune must be performed each day the instrument will be used, prior to casework or QC check analysis. The tune must be performed in the polarity mode(s) used for	At a minimum, attempt the following corrective action if any of the performance checks fail: 1. Repeat test. 2. Troubleshoot using manufacturer's recommendation as outlined in the MassHunter Users Guide. 3. Call for technical

	Instrument	Frequency	Criteria	Corrective Action
	Tox #2 LCMSMS LC Model: Agilent 1260 Infinity Series SN: See instrument maintenance manual for separate components MS Model: Agilent 6420 MS/MS SN: SG17509007		analysis and the tune report must indicate "Pass" for the range of m/z values used for analysis (e.g., for THCA_U: positive ESI tune - "Pass" indicated on tune report for m/z values 118.09 – 622.03). Autotune must be performed monthly or as needed. Once the EMV value registers 2400, the electron multiplier should be changed. Logbooks are located in lab area near equipment. Documentation may be archived in Resource Manager.	support. 4. Tag out of use. 5. Advise lab manager or supervisor and call for a service engineer. 6. Record problem in the instrument maintenance manual or, if necessary, on a Corrective Action Report.
Immunoassay Instruments	Dynex Magellan Biosciences Model: DSX Automated ELISA System SN: 1 DXC-2090	**External:** Refer to specific instrument contract/agreement for appropriate support phone numbers and service agreement Orasure Technologies Inc (800) 869-3538	**External:** Meet external vendor criteria. **Internal:** Daily maintenance as outlined in the technical manual. Dynex Only: A self test must be performed each day the instrument will be used, prior to analysis. Use Weekly/Monthly -and daily Maintenance Forms found in Qualtrax. Logbook is located in lab area near equipment. Documentation may be archived in Resource Manager.	At a minimum, attempt the following corrective action if any of the performance checks fail: 1. Repeat test. 2. Troubleshoot using manufacturer's recommendations. 3. Call for technical support. 4. Tag out of use. 5. Advise lab manager or supervisor and call for a service engineer. 6. Record problem in the instrument maintenance manual or, if necessary, on a Corrective Action Report.

	Instrument	Frequency	Criteria	Corrective Action
GC Headspace Instruments	BA #3 GC Perkin Elmer TurboMatrix 110 Model: Clarus 500 GC Part #: N6519100 SN: 650N6061207 HS SN #: HS110L0606128 BA #4 GC Perkin Elmer TurboMatrix 110 Model: Clarus 500 GC Part #: N6519100 SN: 650N7040903 HS SN #: HS110L0703227 BA #5 GC Perkin Elmer TurboMatrix 110 Model: Clarus 580 GC Part #: N6519580 SN: 580S11033102 HS SN #: HS110L1103283	**External:** Refer to specific instrument contract/agreement for appropriate support phone numbers and service agreement Perkin Elmer Chromatography Division (800) 672-0077 x3292 **Internal:** before use (once/day) and after send-out repair	**External:** Meet external vendor criteria. **Internal:** Refer to Chapter 5.0 Ethanol Analysis by Dual Column Headspace for Batch Acceptance Criteria. **Maintenance(as needed):** Change O-rings, carbide discs, column, and needle. Logbooks are located in lab area near equipment (Internal criteria checks are kept with batch data separate from the instrument logbook). Documentation may be archived in Resource Manager.	At a minimum, attempt the following corrective action if any of the performance checks fail: 1. Repeat test 2. Troubleshoot using manufacturer's recommendations. 3. Call for technical support. 4. Tag out of use. 5. Advise lab manager or supervisor and call for a service engineer. 6. Record problem in the instrument maintenance manual or, if necessary, on a Corrective Action Report.
Pipette Calibration Check System	ARTEL Pipette Tracker® Model: PCS3 Part #: PCS-103 SN: 7-9152	**External:** Every other year Artel® (888) 406-3463 **Internal:** Monthly After send-out repair: Perform a monthly calibration.	**External:** Meet external vendor criteria. **Internal:** Meet external vendor criteria as described in the manufacturer's procedure guide. Logbook is located in Resource Manager.	If monthly calibration verification is not successful, the vendor will be contacted.
Hydrogen Generator	HG #5 Parker Hannifin Model: H2PEM-510-L1466 SN: 12PHG5185	**External:** None **Internal:** Every 6 Months	**Internal:** Maintains pressure and produces hydrogen gas.	If the generator does not meet criteria: 1. Tag out of use.

	Instrument	Frequency	Criteria	Corrective Action
	HG #6 Parker Hannifin Model: H2PEM-510-L1466 SN: 15PHG5058	Parts contained in vendor 6 month maintenance kit should be replaced	Logbooks are located in lab area near equipment. Documentation may be archived in Resource Manager. Hydrogen Generator Water Check forms (document number 13269) are stored in Resource Manager.	2. Troubleshoot using appropriate manufacturer's manual. 3. Advise lab manager or supervisor. 4. Contact manufacturer's technical support.
	HG #9 Swissgas Model: 6920.10.050 HG Basic 500 SN: 0842-0024-2221			
	HG #8 Parker Hannifin Model: H2PEM-100-L1466 SN: 09PHG5126			
	HG #3 Parker Hannifin Model: H2PEM-100-L1466 SN: 11PHG5008			
Nitrogen Generator	NG #1 Peak Scientific Model: NM32LA SN: A16-01-163	**External:** Annually **Internal:** None	**External:** Must meet external vendor criteria. **Internal:** Maintains pressure and produces nitrogen gas. Logbooks are located in lab area near equipment. Documentation may be archived in Resource Manager.	If the generator does not meet criteria: 1. Tag out of use. 2. Troubleshoot using appropriate manufacturer's manual. 3. Advise lab manager or supervisor. 4. Contact manufacturer's technical support.
	NG #2 Peak Scientific Model: NM32LA-A SN: 771040846			

	Instrument	Frequency	Criteria	Corrective Action
Water System	ELGA PureLab Flex 3 Model #: PF3XXXXM1-US Serial #: FLC00008914	**External:** None **Internal:** Purification Filter – replace every 6 -12 months or as needed Pretreatment Filter – replace approximately every 6 months or as needed Vent Filter – replace approximately every 12 months or as needed UV lamp – replace approximately every 18 months or as needed Quartz thimble – replace approximately every 18 months or as needed, typically done with UV lamp For repairs and maintenance: ELGA (877) 315-3542	Meet manufacturer's recommendations	If a water purifier system does not meet the criteria: 1. Take out of service 2. Replace filter(s) and/or UV lamp as necessary 3. Advise Chemistry Laboratory Manager/designee 4. Contact ELGA technical support

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9.0 Title: **APPENDIX - REFERENCES**

Equipment Manuals

The manufacturer's manuals for the following equipment are located within the Toxicology Laboratory:

GC/MS

Agilent 5975 Series MSD Operation Manual, Third Edition, Feb 2010.
Operator's Manual Viva-E Drug Testing System (Corresponding to Software Version No: 2.0)

Operating guide for GC 7890A found here:
https://www.agilent.com/cs/library/usermanuals/Public/G3430-90011.pdf

Quick Reference Guide for GC 7890A

LC/MS/MS

Operating guides are found on the instrument computer at C:\Familiarization\Manuals

DSX

Operator's Manual for DSX Automated ELISA System (For Revelation 6.0 and above)

GCHS

TotalChrom Workstation User's Guide Volume I, Perkin Elmer, February 2001

TotalChrom Workstation User's Guide Volume II, Perkin Elmer, February 2001

TurboMatrix Headspace Sampler and HS 40/110 Trap User's Guide, Perkin Elmer, November 2005

Turbomatrix Headspace Samplers Instrument Manual, Perkin Elmer, April 2000

Clarus 500 GC User's Guide, Perkin Elmer, August 2002

Clarus 500 GC Installation Guide, Perkin Elmer, August 2002

Clarus 500/580 GC User's Guide, Perkin Elmer, February 2010

Clarus 500/580 GC Installation Guide, Perkin Elmer

Pipettes/Diluters

Hamilton User's Manual, Microlab 600 Series, ML600 Basic Manual, Hardware Installation and Basic Operation (Rev.C)

Hamilton User's Manual, Microlab 500A Series (Rev. D)

Eppendorf Series 2000 Reference fixed-volume and adjustable Pipettes Instruction Manual

Eppendorf Repeater stream, Repeater Xstream Operating Manual

Eppendorf Research Instruction Manual

Eppendorf Repeater M4 Manual

MLA Pipette Operator's Manual

Balances

Mettler Toledo Excellence XS Analytical Balances Operation Instructions

Operating Instructions Mettler Toledo PG-S Balances (0.001 g, 0.01 g), 1998

Pipette Calibration

Pipette Calibration System

- Artel PCS3 Procedure Guide
- Artel PCS3 Validation Guide
- Artel Pipette Tracker User Manual (Rev 15S5820D, August 2011)

General References:

OraSure Technologies, Inc., Package Inserts

Immunalysis, Inc., Package Inserts

ANSI/ASB Standard 017, Standard Practices for Measurement Traceability in Forensic Toxicology, First Edition, 2018

ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology, First Edition, 2019

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9.1 Title: APPENDIX - ABBREVIATIONS KEY

Abbreviations Key

⊕, POS - positive
⊖, NEG – negative
6AM, 6-AM, 6-MAM – 6- acetyl morphine
7AC – 7-aminoclonazepam
11-OH-THC - 11-hydroxy- Δ^9-tetrahydrocannabinol
ACQ – acquisition
AG, AGY - agency
ALP- alprazolam
AM, AMP, AMPH – amphetamine
BA – blood alcohol
BAC – blood alcohol concentration
BAK – blood/alcohol kit
B/C - barcode
BENZO, BENZ, BZ, Benzodiazep – benzodiazepines
BLNK, BLK – blank
BUP - buprenorphine
BZE - benzoylecgonine (cocaine metabolite)
CAL – calibrator
CALIB - calibration
CARI, CAR – carisoprodol
CE - cocaethylene
CHROM - chromium
CF – correction factor
CLON, CLN – clonazepam
CoA – certificate of analysis
COC, COCN, C – cocaine
CO, CU, CUT, C/O - cutoff
COD – codeine
CRM – certified reference material
CTRL, CTL – control
CV – coefficient of variation
dAbs/m – delta absorbance per minute
DA – District Attorney
DEF – deferred
DI - deionized
DIAZ – diazepam
DS – drug screen

DS DEF – deferred from drug screening
EBT – evidential breath test
EI – electron ionization
ELISA – enzyme-linked immunosorbent assay
EMIT – enzyme-multiplied immunoassay technique
EMV- electron multiplier voltage
ESI – electrospray ionization
ETHAN, EtOH – ethanol
EV #, EN – event number
EVI – evidence
EXP – expiration
FA – formic acid
FA – further analysis
FA – Forensic Advantage
FEN – fentanyl
FLU - flunitrazepam
FN – first name
GC – gas chromatograph
GCHS – headspace gas chromatograph
GCMS, GC/MS – gas chromatograph/mass spectrometer
GHB – gamma hydroxy-butyrate
HI – high
HYC – hydrocodone
HYM - hydromorphone
IMM – Immunalysis
INJ - injection
INT STD, ISTD, IS – internal standard
KIO - kit individually opened
LC – liquid chromatography
LCMS, LC/MS – liquid chromatography/mass spectrometry
LC/MS/MS, LC/MSMS, LCMSMS– liquid chromatography tandem mass spectrometry
LIMS - Laboratory Information Management System
LN – last name
LOD – limit of detection
LOQ – limit of quantitation
LORAZ, LOR - lorazepam
MDA - 3,4-methylenedioxyamphetamine
MDMA - 3,4-methylenedioxymethamphetamine
MEPRO, MEP - meprobamate
METH, MAMP – methamphetamine
MG - mitragynine
MOR – morphine
MPH - methylphenidate
MRM – multiple reaction monitoring
MSD – mass selective detector
MTD - methadone
N-PROP – n-propanol
N/A – not applicable

NA, NarcAn – narcotic analgesic
NBUP - norbuprenorphine
NCAL – negative calibrator
NFA – no further analysis
NM - name
NORDIAZ, NORD – nordiazepam
NT, NIT - nitrate
OH-ALP - α-hydroxyalprazolam
OH-TRIAZ - α-hydroxytriazolam
OD – optical density
ODT – o-desmethyltramadol
OF – oral fluid
OR - object repository
ORS - OraSure
OPI, OP, OPIA – opiates
OX - oxidant
OXAZ – oxazepam
OXC, OXY, OXYC - oxycodone
OXM, OXYM – oxymorphone
P# - LVMPD personnel number
PBT – preliminary breath test
PC - Property Connect
PCP - phencyclidine
PC Sgt. - Property Crimes Sergeant
PFAA - pentafluoropropionic acid anhydride
PFTBA – perfluorotributylamine
PHEN - phentermine
PI – personal identifiers
PN – part number
POI - persons of interest
PREP – preparation
PSI – pounds per square inch
PTFE – polytetrafluoroethylene
QC – quality control
QNS – quantity not sufficient
QS – quantity sufficient
REQ - request
RFLE - Request for Forensic Laboratory Examination
RGT – reagent
RM – Resource Manager
RPT – repeated
RRT – relative retention time
RT – retention time
SD – standard deviation
SEP – separation
SDS - safety data sheet
SOLN, SLN – solution
SPE – solid phase extraction

SG, SPGR, SP.GR. – specific gravity
SN – serial number
S/N – signal to noise
STD – standard
SU – suspect
SVT – specimen validity testing
TARG - target
TEMAZ, TEM – temazepam
TFE - tetrafluoroethylene
T, THC - Δ^9-tetrahydrocannabinol
THCA - 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (Marijuana metabolite)
TIC – total ion chromatogram
TOX – toxicology
TRM - tramadol
TRIAZ – triazolam
UAC – urine alcohol concentration
UC – until consumed
UoM, MU – measurement uncertainty
UR - unit record, urine
V- volts, volume
VOL - volume
WB – whole blood
WBC – whole blood control
XTC, EX – ecstasy (see MDMA)
ZAL – zaleplon
ZOL – zolpidem
ZOP - zopiclone

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9.2 Title: APPENDIX – SOFTWARE VERSIONS

Toxicology
Computer Software Versions

INSTRUMENT	INSTRUMENT NAME	SOFTWARE VERSION
GC/MS	**Tox #9, Tox #10**	Chemstation E.02.01.1177
LC/MS/MS	**Tox #1**	MassHunter Workstation Data Acquisition: B.08.00 Quantitative Analysis: B.07.01 Qualitative Analysis: B.07.00
	Tox #2	MassHunter Workstation Data Acquisition: B.08.02 Quantitative Analysis: B.08.00 Qualitative Analysis: B.08.00
GC/HS	**BA #3 GC, BA #4 GC, BA #5 GC**	TotalChrom Workstation 6.3.2.0646
Immunoassay	**Dynex DSX ELISA**	Revelation: v.6.24
Pipette Calibration System	**ARTEL Pipette Tracker®**	Pipette Calibration System v1.4.2