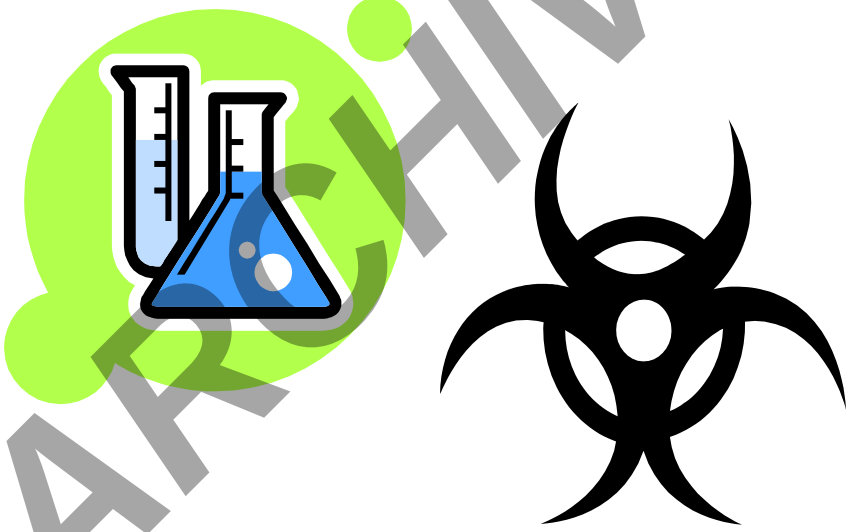
Las Vegas Metropolitan Police Department
Forensic Laboratory
5605 W. Badura Ave. Ste. 120B
Las Vegas, NV 89118

Toxicology Technical Manual



0.0 Title: TABLE OF CONTENTS

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 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 Chapter 5.8, Handling of Evidence (Test and Calibration Items), in the LVMPD Forensic Handbook. After evidence has been data entered into ACE (Active Control of Evidence), 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. An “internal move” to the Toxicology refrigerator location in ACE 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.

2.2 Evidence

2.2.1 Evidence Description

Blood Kit

A standard blood kit is a white approximately 5” x 3” x 1 ½ ” cardboard box with Las Vegas Metropolitan Police Forensic Lab printed on the side. 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 2.8 Reporting)

2.2.2 Subject Name

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 ACE label. This will be used as the name on the Forensic Laboratory Report of Examination. 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 ACE, but if they appear on the ACE label and match the information on the blood kit they can be used on the Forensic Laboratory Report of Examination; the name in ACE 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)

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. If the nature of the sample is such that testing can be outsourced, the option will be discussed with the agency. Agency contact information is located at

H:\CB\Forensics\Toxicology\Contacts. The communication will be documented in the case file.

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 Exception

If no drug screen was requested but the blood alcohol result is less than 0.085 g/100 mL, the case is referred to Drug Screening for a standard drug screen.

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.

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 analyzed according to Chapter 3.1 EMIT Urine Screening Procedure.

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.

Because lorazepam has low cross-reactivity to the urine drug screen, samples from Grand Larceny ("Trick Roll") and Drug Facilitated Sexual Assault cases will be confirmed for benzodiazepines regardless of the screening results.

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.

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, 4.1 (procedures for blood), and 4.2 (procedures for urine).

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, samples will be outsourced only when all in-house results are negative.

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.

2.4 Dates of Testing

2.4.1 Start Date

"Start date of testing" is defined as the date the examiner transfers the evidence into his/her custody in the LIMS.

In the event that the analyst did not transfer the evidence into his/her custody in the LIMS prior to testing (e.g., the LIMS is unavailable), the analyst will document in their case notes when analysis began.

When start date of testing is included on the Laboratory Report of Examination, the start date of testing for Drug Screening/Confirmation reports will be the start date of drug screen testing.

2.4.2 End Date

"End date of testing" is defined as the distribution date on the Laboratory Report of Examination.

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 examiner 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, urine drug screens, 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
- EMIT QC Packet 041017 DR
- THCB QC Packet 041017 GCMS#9 SW

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 Procedures and 3.1 EMIT Urine Screening Procedures 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 Data Not Used

Data that is generated but not reported will be labeled with "data not used," the reason the data is not used, and the initials and date of the analyst. If the data for a batch or partial batch of samples is not used, the reason may be documented on only the first page of the QC packet of the batch if all the rejected data is contained within the QC packet.

2.8 Reporting

2.8.1 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 – "unable to determine due to insufficient quantity of blood/urine"
- Substance interferes with analyte of interest – "unable to determine due to interference"

2.8.2 Evidence Description

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

2.8.2.1 Blood

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

- "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 footnote on the report. If more than one blood tube contained blood it will be noted which blood tube was used for analysis. For example:

- "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."
- "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:

- "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."

2.8.2.2 Urine

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

- "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 footnote on the report.
For example:

- "A metal can containing a plastic conical tube of urine was received."

2.9 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.

2.10 Department Drug Testing

Except as indicated, the above rules for evidence handling and workflow will apply to Department Samples.

2.10.1 Department Drug Testing samples will be handled as outlined in the LVMPD Department Manual section 5/110.01 – *General Fitness for Duty and a Drug Free Workplace*.

2.10.2 Department Drug Testing samples, including random (RA), reasonable suspicion (RS), pre-employment (PE), transfer (TR), recruit (patrol academy (ACA) or corrections academy (DSD)), voluntary (VO), and samples from commissioned supervisors collected by the Critical Incident Review Team (CIRT) are delivered to the Forensic Laboratory. These samples are logged into a spreadsheet by laboratory staff. The samples are stored in a Toxicology refrigerator and will have labels placed upon the urine sample trays to display the status of a batch.

2.10.3 Flaws

Samples will be rejected for testing for the following flaws:

- Box not checked "Yes" for temperature check
- Specimen ID on seal and chain-of-custody form do not match

- Donor's initials are missing from seal
- Seal is broken
- Insufficient sample volume to complete testing

The following flaws may be corrected with a memo from the collector if the collector has retained sufficient information:

- The collector's signature and printed name are missing on form
- No check mark for how donor identified

2.10.4 "Start date of analysis" for Department samples urine drug screens will be documented on the QC packet.

2.10.5 Department samples that confirm positive for drugs, alcohol, and/or adulterants, and all samples from commissioned supervisors (CIRT) are considered evidence. They are entered into ACE and stored frozen until they are transferred to the Evidence Vault.

2.10.6 The following types of samples will be entered into LIMS

- All CIRT samples
- Any sample that requires a drug confirmation
- Any RS sample on a drug screen batch or blood alcohol batch analyzed utilizing LIMS

2.10.7 Disposal

Department samples that are negative (excluding those from commissioned supervisors (CIRT) and RS samples) will be disposed of in a biohazard bin as soon as practicable after the final report has been distributed. RS samples will not be disposed of until instructed to do so by the Toxicology Manager/designee.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

3.0 Title: ELISA BLOOD SCREENING PROCEDURES

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, Temazepam, and Triazolam
Cannabinoids	10 ng/mL	THC Carboxylic Acid
Carisoprodol	500 ng/mL	Carisoprodol and Meprobamate
Cocaine	50 ng/mL	Benzoylecgonine
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, calibrators, 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 6.6.1.1 for QC requirements. Store in the freezer. After thawing, store in the refrigerator)

Drug Solutions (See Chapter 3.3 for preparation instructions and Section 6.4 for QC requirements. Store in the freezer.)

- Blood Screen Working Solution (Calibrator 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 in the refrigerator)

- 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, kit calibrators, reagents, working solutions, and whole blood from the refrigerator and 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.

Calibrators and Positive Control Preparation

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

The positive control and calibrators must be freshly prepared in blood the same day as casework samples on the batch.

Calibrators 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
Calibrator Blank	0 µL	1 mL	1 mL
Calibrator Low	10 µL	1990 µL	2 mL
Calibrator Cutoff	20 µL	1980 µL	
Calibrator High	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, rack position 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, calibrators 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

Calibrators 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 calibrator must be greater than the mean OD of the low calibrator which must be greater than the mean OD of the cut-off calibrator which must be greater than the mean OD of the high calibrator (i.e., blank>low>cut-off>high).
- There must be at least 0.05 separation between the mean OD values of all four calibrators, without all positive calibrators resembling blank sample OD values.
- Individual calibrator OD data readings must not overlap with other calibrators.
- The OD values of the cut-off calibrator must result in a coefficient of variation (CV) of < 20%.
- All positive controls must be reported as positive.
- Kit Calibrators are analyzed on each plate (Kit1/Kit2). They are used to track plate performance trends, not to set a calibration curve. Kit Calibrator results should be consistent with historical results.

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

It is noted that even though all acceptance criteria are met within a batch, the drug screen analyst must rely on his/her 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 calibrator and the negative calibrator. These values are an average of two results generated for each calibrator. 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, calibrators, and controls must be capped and placed in the refrigerator. The calibrators and controls must be analyzed the same day the samples are analyzed. A new Batch Sheet and Sample Caddy Load List will need to be prepared the day the batch is 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	✓	✓

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 cutoff will be reported as negative; samples with O.D. values less than or equal to the cutoff will be reported as positive.

NOTE: Blood samples from commissioned supervisors (CIRT) will only be screened using the cannabinoid, cocaine, methamphetamine, opiate, and phencyclidine plates. See LVMPD Department Manual section 5/110.01 – *General Fitness for Duty and a Drug Free Workplace* for further information.

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3.1 Title: EMIT URINE SCREENING PROCEDURE

Purpose and Scope

This procedure is intended to qualitatively determine the presence of seven analytes/classes of drugs in biological urine samples received into the laboratory, utilizing Enzyme-Multiplied Immunoassay Technique (EMIT). The classes, their specific analytes, and their cut-off concentrations are:

Drug Class	Cut-off Concentration	Specific Analytes
Amphetamines	500 ng/mL	Amphetamine and Methamphetamine
Benzodiazepines	200 ng/mL	Nordiazepam, Oxazepam, Temazepam, α-Hydroxyalprazolam and α-Hydroxytriazolam
Cannabinoids	50 ng/mL	THC Carboxylic Acid
Cocaine	150 ng/mL	Benzoylecgonine
Ecstasy	300 ng/mL	Methylenedioxymethamphetamine (MDMA)
Opiates	2,000 ng/mL	Morphine, Codeine, and Hydrocodone
PCP	25 ng/mL	Phencyclidine

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

Principle

The EMIT (Enzyme Multiplied Immunoassay Technique) II Plus Assay is a homogenous enzyme immunoassay technique used for the analysis of specific drug analytes. The assay is based on competition between drug in the specimen and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the specimen can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically at 340 nm. The absorbance is directly proportional to the amount of drug present. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacteria (*Leuconostoc mesenteroides*) enzyme employed in the assay.

Instrumentation

The instrument used for the analysis is a Siemens Healthcare Diagnostics Inc. EMIT Analyzer Viva-E model. 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 consist of the following:

- Worklist Orders Report
- Load List
- Controls Report
- Quality Controls Report
- Calibration Report
- Blank Rotor Results
- Reagent Info
- Calibrators and Controls Lot Numbers
- PDF files of the Viva-E analysis results

The QC Packet is maintained in Qualtrax.

Materials

- Plastic Disposable Transfer Pipettes
- 5 mL-13 x 75 mm plastic frosted test tubes
- 2 mL Pediatric Sample Cups
- Pediatric Sample Cup Adapters

Reagents

Negative Urine (See Section 6.6.2.1 for QC requirements. Store in the refrigerator)

Urine Screen Working Solutions (See Chapter 3.3 for preparation instructions and Section 6.4 for QC instructions. Store in the freezer.)

- Urine Screen Working Solution (Calibrator and Control):
  - 7.5 µg/mL Benzoylecgonine, 15 µg/mL MDMA, 25 µg/mL Methamphetamine, 100 µg/mL Morphine, 10 µg/mL Oxazepam, 1.25 µg/mL PCP, 2.5 µg/mL THCA in methanol

Chemicals (store at room temperature)

- System Solution
- 0.1 M Sodium Hydroxide Solution
- Sodium Hypochlorite Solution
- 0.1 M Hydrochloric Acid Solution

Substrate/Enzyme Reagents (store in the refrigerator)

- Cannabinoid Assay
- Ecstasy Assay
- Cocaine Metabolite Assay
- Phencyclidine Assay
- Opiate Assay
- Benzodiazepine Assay
- Amphetamines Assay

Validity (Adulterants) Reagents (store in the refrigerator)
- Oxidant Validity Test
- Creatinine Validity Test
- Nitrite Validity Test
- Specific Gravity Validity Test
- pH Validity Test

Calibrators and Controls (store in the refrigerator)
- Negative Drug Control: Level 0
- High Drug Control: Level 5
- 6 AM/Ecstasy Drug Control: Level 4
- Negative Validity Calibrator
- Adulterant Validity Controls: Levels 1, 2, 3 & 4
- Chromium Validity Calibrator: Level 50
- Chromium Validity Control: Level 100
- Creatinine Validity Calibrators: Levels 2 & 20
- Specific Gravity Validity Calibrators: Levels 1.0030 & 1.0200
- pH Validity Calibrators: Levels 2.0, 3.0, 4.5, 9.0, 11.0 & 12.0
- Nitrite Validity Calibrator: Level 500

Reagent QC

Except for the in-house prepared Urine Screen Working Solutions and negative urine, all reagents are commercially prepared. These reagents are QC'ed concurrently with use. All passing criteria for a batch (see **Acceptance Criteria**) must be met.

Calibrator Preparation

1) Allow working solution to equilibrate to room temperature prior to pipetting.
2) Add 100 µL of the Urine Screen Standard Working Solution to a 5 mL Class A volumetric flask. QS to 5 mL with negative urine and mix. Prepare fresh daily for one time use. Variations to the formulations must be approved by the Forensic Toxicology Manager or designee.
3) Using a disposable transfer pipette, aliquot a minimum of 0.25 mL of sample into a pediatric sample cup.

Positive Control Preparation

1) Allow working solution to equilibrate to room temperature prior to pipetting.
2) Add 150 µL of the Urine Screen Control Working Solution to a 5 mL Class A volumetric flask. QS to 5 mL with negative urine and mix. Prepare fresh daily for one time use. Variations to the formulations must be approved by the Forensic Toxicology Manager or designee.
3) Using a disposable transfer pipette, aliquot a minimum of 0.25 mL of sample into a pediatric sample cup.

Sample Preparation

1) Remove samples from the refrigerator and allow to equilibrate to room temperature.
2) For split Department samples, separate D and E sample cups, using D samples for analysis.
3) Prior to analysis, ensure the urine sample to be analyzed has the following information placed upon it: Lab number/item number (casework only), analyst's initials, and sample position.
4) Create a Worklist in either LIMS or WinTox6. Order a positive control prior to case/Department samples, after every 10 samples throughout the batch, and at the end of the run.
5) Using the Worklist create a Loadlist in the Viva-E software. The Loadlist must be included in the QC Packet, unless WinTox software is used
4) Using the Worklist, label 13x75 mm test tubes with Sample ID and position.
5) Using a disposable transfer pipette, aliquot a minimum of 0.5 mL of sample into 13x75 mm test tube. Pediatric sample cups and pediatric sample cup adapters may be used for limited volume samples (i.e., < 0.5 mL), and must contain a minimum of 0.25 mL of solution.

Procedure

1) Calibrate the instrument per Test Panel requirements.
2) Ensure calibration meets criteria specified below (see Calibration).
3) Load samples and positive controls on the sample rotor wheel, verifying positions with the Loadlist/Worklist.
4) Analyze samples and positive controls.
5) Evaluate results using acceptance criteria below (see Sample Batch).

Test Panels

Several sample types are analyzed in the Toxicology Detail, including Department samples and legal casework (forensic) samples. Each of these sample types have specific drug classes and/or adulterant tests which must be performed. The table below details the classes and adulterant tests needed.

Department Samples Reasonable Suspicion **(RS)** Pre-Employment **(PE)** Commissioned Academy **(ACA)** Corrections Academy **(DSD)** Voluntary **(VO)** **Negative Urine** **7-Panel + Adulterants**	**Forensic Samples** **In-house Positive Control** **7-Panel**	**Department Samples** Random **(RA)** Transfer **(TR)** **CIRT** **6-Panel + Adulterants**
Cocaine	Cocaine	Cocaine
Ecstasy	Ecstasy	Ecstasy
Amphetamines	Amphetamines	Amphetamines
Opiates	Opiates	Opiates
PCP	PCP	PCP
THC-carboxylic acid	THC-carboxylic acid	THC-carboxylic acid
Benzodiazepines	Benzodiazepines	
Adulterants		Adulterants

LIMS

Department sample drug screening need not be completed in LIMS. Department samples that screen positive will be entered into the LIMS, the results will be hand-entered, and the paperwork generated shall then be uploaded into the Object Repository. QC Packets will be stored in Qualtrax.

Acceptance Criteria

Calibration

Prior to analyzing samples and reporting out results based on the screening method, the standard/calibrator results will automatically be evaluated by the software. Only after the standards/calibrators have met passing criteria will samples be analyzed on the instrument. In addition, the difference between the replicate measurements for each calibrator cannot be > 0.015 dAbs/m.

Review the control values in the Cut Neg/Sep and Hi Cut/Sep columns on the Quality Controls Report. The actual separation (Cut Neg and Hi Cut) must be greater than the expected separation (Sep). Expected separation values are located at H:\CB\Forensics\Toxicology\SCREENS\Urine Screens--VIVA-E\EMIT Assay Separations

- If a control fails it can be rejected and remeasured. If it fails a second time, the drug assay will be recalibrated.

Sample Batch

Ensure that the in-house controls are recorded as **POSITIVE** by the software throughout the batch.

- If a **POSITIVE** result is not obtained for one of these in-house controls, the samples/specimens bracketed by valid controls prior to and following the failed control, must be reanalyzed for that drug/class in a future batch. If more than one control fails to give a **POSITIVE** result, the entire batch is invalid and must be reanalyzed for that drug/class.

Confirm that results were obtained for all tests for all samples.

- If any sample needs to be repeated (e.g., "rejected" appears as the result) the result can be rejected and measured again on the same day. If results are not obtained a second time, the sample should be repeated on the next calibrated batch.

It is noted that even though all acceptance criteria are met on a batch, the drug screen analyst must rely on his/her 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.

Specimen Validity Testing

The Department Drug Testing program requires a urine sample to be tested for indications of adulteration and substitution. A preliminary test gives a qualitative determination as to whether the sample is consistent with human urine (whether the sample has been substituted with other liquids) and whether the sample contains commercially available adulterants or other common household items. This is done primarily by the Siemens Syva EMIT Viva-E Analyzer. Samples that give abnormal results for creatinine and specific gravity are suspected of substitution. Proof of substitution requires both the creatinine to be below 2 mg/dL *AND* the specific gravity to be ≤ 1.0010 or ≥ 1.0200. Samples that give abnormal results for pH, Nitrites or Oxidants are suspected of adulteration. Proof of adulteration requires a pH of < 3 or ≥ 11, Nitrites of ≥ 200 µg/mL OR Oxidants of ≥ 50 µg/mL.

- If a sample is suspected of either substitution or adulteration, it will first be repeated on a different calibrated batch. If the sample still gives abnormal results indicating substitution or adulteration, further testing may be required.

Reporting

The Viva-E software automatically evaluates the absorbance value of the sample by comparing it to the absorbance value of the cutoff. Samples with absorbance values greater than or equal to the cutoff are reported as positive. Samples with absorbance values less than the cutoff are reported as negative. Negative reports will be issued through the LIMS for casework samples. Negative Department samples will be reported in memo format to the Health Detail.

Enter results for all Department samples into the Tox spreadsheet found at H:\CB\Forensics\Toxicology\SCREENS\Dept Sample & Casework Spreadsheets.

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3.2 Title: EMIT REQUIRED MAINTENANCE

Record daily and weekly maintenance on the s10056_Viva-E_Daily_WklyChecklist form 5410.

There is no end-of-day procedure that must be followed. Before going into standby mode the instrument will automatically clean the reagent needle and the sample needle.

Daily Maintenance

Daily maintenance is to be completed prior to running samples or controls.

- Fill water container if low with system liquid and distilled water (25 mL system liquid per 10 L water)
- Empty waste container if full or if running a batch will fill up the waste containers
- Check cuvette rotor blank results (results should be less than 0.0200) and replace rotor if necessary
- Fill HCl bottle in the reagent rotor with 0.1 M HCl
- Fill tube in W position of sample rotor with sodium hypochlorite solution
- Remove cuvette cover, visually check wash arm and cuvette rotor, and manually check mixers/mixer belts
- Make sure the cooling unit is on and operating correctly
- Fill system

Weekly Maintenance

Weekly maintenance will be performed when the instrument is in use.

Perform a needle rinse. Fill the needle rinse and HCl bottles on the reagent rotor and place a tube filled with fresh sodium hypochlorite in position W on the sample rotor. From the main menu select “special functions”, then “rotor systems”, then double click “rotor/needle rinse” and select “needle rinse”.

Check syringes and Teflon tips for air bubbles and leakage. Clean or replace syringes if necessary.

Monthly Maintenance

Rinse water and waste containers with 0.1 M NaOH to clean. Afterward, rinse several times with distilled water to remove residue.

Quarterly Maintenance

- Replace mixer belt
- Replace water filter

- Replace drying block on wash arm

Semi-Annual Maintenance (performed by a field service engineer)

- Perform a system clean and rinse
- Perform Quarterly Maintenance

As Needed Maintenance

- Replace cuvette rotor (every 10,000 tests or after SD errors)
- Replace lamp
- Fill cooling unit with cooling liquid (store at room temp)

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3.3 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. Calibrators and controls are prepared from different manufacturers (e.g., Cerilliant used for calibrators, Cayman Chemical used for controls).
2. THCA stock solutions must be derived from (-)-11-nor-9-Carboxy –Δ9-THC (e.g., Cerilliant item number T-018).
3. Methamphetamine stock solutions must be derived from S(+)-Methamphetamine (e.g., Cerilliant 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 calibrator and control containing THCA.

Volume to Pipette	Stock Solution	GC Grade Methanol Volume	Final Concentration
500 µL	100 µg/mL Benzoylecgonine	QS to 10 mL	5 µg/mL
500 µL	1.0 mg/mL Carisoprodol		50 µg/mL
200 µL	100 µg/mL d-Methamphetamine		2 µg/mL
100 µL	100 µg/mL Morphine		1 µg/mL
250 µL	100 µg/mL Oxazepam		2.5 µg/mL
100 µL	100 µg/mL Oxymorphone		1 µg/mL
1000 µL	10 µg/mL Phencyclidine		1 µg/mL
1000 µL	10 µg/mL THC-carboxylic acid		1 µg/mL

Quality Control:
See section **6.4 Quality Control Checks of Drug Stock and Working Solutions** for Quality Control procedures.

Urine Screen Working Solutions

Preparation:

1. Calibrators and controls are prepared from different manufacturers (e.g., Cerilliant used for calibrators, Cayman Chemical used for controls).
2. THCA stock solutions must be derived from (-)-11-nor-9-Carboxy –Δ9-THC (e.g., Cerilliant item number T-018).
3. Methamphetamine stock solutions must be derived from S(+)-Methamphetamine (e.g., Cerilliant 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 amber vials must be used to store working solutions

Volume to Pipette	Stock Solution	GC Grade Methanol Volume	Working Solution Concentration
750 µL	100 µg/mL Benzoylecgonine	QS to 10 mL	7.5 µg/mL
1500 µL	100 µg/mL MDMA		15 µg/mL
250 µL	1.0 mg/mL Methamphetamine		25 µg/mL
1000 µL	1.0 mg/mL Morphine		100 µg/mL
1000 µL	100 µg/mL Oxazepam		10 µg/mL
125 µL	100 µg/mL Phencyclidine		1.25 µg/mL
250 µL	100 µg/mL THC-carboxylic acid		2.5 µg/mL

Quality Control:
See section **6.4 Quality Control Checks of Drug Stock and Working Solutions** for Quality Control procedures.

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

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 his/her 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.

4.0.4.1 Linearity

Each batch shall be calibrated on calibration standards specified in chapters 4.1 and 4.2. The calibration standards shall be extracted on the same day as the casework samples. 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. An r^2 value of greater than or equal to 0.995 must be achieved.

4.0.4.2 Controls

All methods shall include control samples, if commercially available. Blood methods may use urine control material diluted with negative blood. 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. If a positive control fails, the case samples bracketed by the two valid controls immediately before and after the failed control will be repeated. If more than one positive control fails, all samples must be repeated.
Controls shall not be derived from the same lot as the calibration standards. If the glucuronide control from a codeine/morphine urine, benzodiazepine urine, or THCA urine batch fails, all samples must be repeated.

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 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 4.0.6.1, 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. 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 ion and at least one qualifying ion. Internal Standards must have a primary ion (a qualifying ion is not required).

4.0.5.3 Ion ratios

Qualifying ion ratios generally should be no greater than ±20 % (for EI and MS/MS methods) or ±30 % (for CI methods) of the ion ratios of the corresponding control or calibrators. However, it is recognized that some ion ratios are concentration dependent and that comparison to a calibrator or control of similar concentration may be necessary, 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. Peaks integrated manually will be reviewed by a second member of staff who is authorized to perform technical reviews on confirmation casework. 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 changes to the system have been made (e.g., injector maintenance, column trim, 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."

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, casework and Department samples analyzed directly after those with concentrations exceeding the carryover check level must be re-injected to confirm 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 must be re-injected to confirm results, if the result is being reported.

4.0.8 Screen Records Review

Confirmation analysts will review all drug screen examination records for each case they analyze and notate "Reviewed" with their initials and date on the screen worksheet.

4.0.9 Reporting

A report that is issued represents a summary of the analytical findings, identifies the substance(s) tested, and lists the amount (usually ng/mL) or "none detected" if no substance is detected at or above the cut-off (see Chapter 4.2.01 for special reporting criteria for methamphetamine in urine). Quantitative results 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. Results may be reported at a concentration of up to twenty percent greater than the highest standard if the method has been validated to be linear at that level.

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 the casework sample is urine, the casework sample can be reported as greater than the highest calibration standard times the dilution factor. (Example: The highest calibration standard is 1000 ng/mL. The casework

sample result is 6514.3 ng/mL and the dilution factor is 5. The result may be reported as greater than 5000.0 ng/mL).

- 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.

4.0.10 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.

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4.1.01 Title: CONFIRMATION - AMP / METH / MDA / MDMA IN BLOOD

Purpose and Scope: This procedure is intended to quantitatively determine amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), and methylenedioxymethamphetamine (MDMA) in blood using a pentafluoropropionic acid anhydride (PFAA) derivative. **Note:** PFAA does not convert pseudoephedrine and ephedrine to methamphetamine in appreciable amounts.

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.

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*
- Autosampler vials, inserts, and TFE faced caps

* Other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Ethyl acetate
- Methanol
- Ammonium hydroxide
- Pentafluoropropionic acid anhydride (PFAA)

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- 1% Acidic methanol
- Phosphate buffer, 100 mM, pH 6.0
- Acetic acid, 100 mM
- Eluting Solution - ethyl acetate containing 2% ammonium hydroxide (pH 12).
  Add additional ammonium hydroxide as necessary to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution – 20 µg/mL of Amp-D_{11} / Meth-D_{14} / MDA-D_5 / MDMA-D_5 in methanol
- Drug working solution – 10 µg/mL Amp/Meth/MDA/MDMA in methanol

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 ng/mL	Amp/Meth/MDA/MDMA Working Solution 10 µg/mL	Volume Whole Blood
1	100	10 µL	990 µL
2	250	25 µL	975 µL
3	500	50 µL	950 µL
4	1000	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of (20 µg/mL) internal standard mix to each tube.
3. Add 4 mL of 100 mM Phosphate Buffer (pH 6.0) to each tube while vortexing.
4. Centrifuge each tube for 10 min at ~3000 rpm.

Solid Phase Extraction:

5. Place the Cerex Clin II SPE columns into an extraction manifold.
6. Load samples and run through SPE columns
7. Wash the SPE columns as follows:
   a. 3 mL distilled water
   b. 2 mL 100 mM acetic acid
   c. 2 mL methanol
   d. 2 mL ethyl acetate
8. Dry SPE columns for at least 15 minutes at ≥20 psi under a gentle steam of nitrogen.
9. Place labeled 16x100 mm screw top glass culture tubes under each SPE column and elute with 2 mL of ethyl acetate containing 2% ammonium hydroxide (pH=12).
10. Add 200 µL of 1% acidic methanol to each tube and vortex.

11. Transfer the tubes to an evaporator bath and evaporate to dryness at 30 ˚C under a gentle stream of nitrogen. **Do not over dry because the amphetamine compounds are volatile.**

Derivatization:

12. Add 50 µL of PFAA and 50 µL of ethyl acetate to each tube and cap. Incubate at 70 C for 30 minutes. Remove from heat and allow the samples to cool.
13. Transfer tubes to the evaporator bath and again evaporate to dryness at 30 ˚C under a gentle stream of nitrogen.
14. Reconstitute with 50 µL of ethyl acetate. Transfer contents to GC/MS autosampler vials/inserts, cap and transfer samples to an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, i.e. DB-5)
Acquisition method: METH_B.M
Data analysis method: METH_B.M
GC parameters:

Injector temp: 250 C
Thermal Aux 2 (Transfer Line): 280 C
MS Source: 230 ºC
MS Quad: 150 C
Oven program: 70 C for 1 min, 20 C/min to 280 C, hold at 280 C for 3 min.
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM) : Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Amphetamine-D_{11}	194	128
Amphetamine	190	118, 91
Methamphetamine-D_{14}	211	163
Methamphetamine	204	160, 118
MDA-D_5	330	167
MDA	325	190, 162
MDMA-D_5	208	344
MDMA	204	339, 160

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4.1.02 Title: CONFIRMATION – COCAINE / BENZOYLECGONINE IN BLOOD

Purpose and Scope: This procedure is intended to quantitatively determine cocaine and benzoylecgonine (cocaine metabolite) in blood.

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

Materials:

- SPE columns (UCT Clean Screen ® CSDAU206, 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:

- Methanol
- Distilled water
- Ethyl acetate
- Ammonium hydroxide
- BSTFA with 1% TMCS

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Hydrochloric acid, 0.1 M
- Phosphate buffer, 100 mM, pH 6.0
- Eluting solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as necessary to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution – 10 µg/mL of Cocaine / BZE in methanol
- Internal standard working solution - 10 µg/mL Cocaine-D_3 / BZE-D_3 in methanol

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 ng/mL	COC/BZE Working Solution 10 µg/mL	Volume Whole Blood
1	50	5 µL	995 µL
2	100	10 µL	990 µL
3	500	50 µL	950 µL
4	1000	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of (10 µg/mL) internal standard solution to each tube.
3. Add 4 mL of 100 mM phosphate buffer (pH 6.0) to each tube while vortexing.
4. Centrifuge tubes for 10 minutes at ~3000 rpm.

Solid Phase Extraction:

5. Place SPE columns into an extraction manifold.
6. Condition SPE columns as follows:
   a. 3 mL methanol
   b. 3 mL distilled water
   c. 1 mL 100 mM phosphate buffer (pH 6.0)
7. Load samples and run through SPE columns.
8. Wash SPE columns as follows:
   a. 3 mL distilled water
   b. 2 mL 0.1 M hydrochloric acid
   c. 3 mL methanol
9. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
10. Place 16 x 100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/isopropanol/ammonium hydroxide solution (78/20/2, pH = 12).
11. Transfer tubes to an evaporator bath and evaporate to dryness at 40 °C under a gentle stream of nitrogen.

Derivatization:

12. Add 50 µL of ethyl acetate and 50 µL of BSTFA with 1% TMCS to each tube and cap. Incubate at 70 °C for 20 minutes. Remove from heat and allow the samples to cool.
13. Transfer contents to GC/MS autosampler vials/inserts, cap and transfer samples to an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent Technologies 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent)
Acquisition method: COC_B.M
Data analysis method: COC_B.M
GC parameters:

Injector Temp: 250 C
Thermal Aux 2 (Transfer Line): 300 C
MS Source: 230 C
MS Quad: 150 C
Oven program: 100 C for 0.25 min, 35 C/min to 220 C, 20 C/min to 300 C, hold at 300 C for 4 min.
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters(SIM) : Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Cocaine-D_3	185	201
Cocaine	182	198, 303
Benzoylecgonine-D_3	243	259
Benzoylecgonine	240	256, 361

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 analytes 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 4.3 for preparation and storage instructions. See Section 6.4 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. Prepare calibrators and controls as described above. Pipet 1.0 mL of each casework blood specimen into a labeled 16 x 100 mm silanized glass screw-top tube.
2. Add 100 µL of internal standard working solution to each tube.
3. Add 2 mL of water to each tube and vortex.
4. Add 800 µL of 10% acetic acid and vortex.
5. Add 6 mL of 9:1 hexane:ethyl acetate solution, cap, vortex, and rock/rotate tubes for 20 minutes.

6. Centrifuge at ~3000 rpm for at least 30 minutes.
7. Transfer the upper organic layer to appropriately labeled silanized tubes.
8. Evaporate samples under nitrogen to dryness at ~30˚ C.

Reconstitution:

9. Add 50 µL of acetonitrile, LC-MS grade to each tube and vortex.
10. Add 50 µL of water, LC-MS grade to each tube and vortex.
11. 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 LCMS
Instrument Make/Model	Agilent 6420 Triple Quadrupole LC/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

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.1.04 Title: CONFIRMATION - CODEINE / MORPHINE IN BLOOD

Purpose and Scope: This procedure is intended to quantitatively determine codeine and morphine in blood.

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

Materials:

- SPE columns (UCT Clean Screen CSDAU206, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes *
- GC/MS autosampler vials, inserts, and TFE faced caps

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Methanol
- BSTFA with 1% TMCS
- Methylene chloride
- Isopropanol
- Ammonium hydroxide

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Phosphate buffer, 100 mM, pH 6.0
- Acetate buffer, 100 mM, pH 4.5
- Eluting Solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution - 10 µg/mL COD/MOR in methanol.
- Internal standard working solution - 2 µg/mL COD - D_3 / MOR - D_3 in methanol.

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	Codeine / Morphine Final Concentration ng/mL	Codeine / Morphine Working Solution 10 µg/mL	Volume Whole Blood
1	50	5 µL	995 µL
2	100	10 µL	990 µL
3	200	20 µL	980 µL
4	400	40 µL	960 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of 2 µg/mL internal standard to each tube.
3. Add 4 mL of 100 mM phosphate buffer and vortex. Centrifuge for 10 minutes at ~3000 rpm.

Solid Phase Extraction:

4. Place SPE extraction columns into an extraction manifold.
5. Condition SPE columns as follows:
    a. 3 mL methanol
    b. 3 mL distilled water
    c. 1 mL 100 mM phosphate buffer, pH 6.0
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
    a. 2 mL distilled water
    b. 2 mL 100 mM pH 4.5 acetate buffer
    c. 3 mL methanol
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Place labeled 16x100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/isopropanol/ammonium hydroxide (78/20/2, pH=12).
10. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40°C under a gentle stream of nitrogen.

Derivatization:

11. Add 50 µL of BSTFA to each tube and cap. Vortex and incubate at 70 °C for 30 minutes. Allow to cool.
12. Transfer contents to GC/MS autosampler vials/inserts, and cap. Place samples on an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C mass spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent)
Acquisition method: Opiates_B.M
Data analysis method: Opiates_B.M
GC parameters:
Injector temp: 250 ºC
Thermal Aux 2 (Transfer Line): 280 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 100 ºC for 0.25 min, 30 ºC/min to 280 ºC hold at 280 ºC for 7.25 min.
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Codeine-D_3	374	346
Codeine	371	343, 234
Morphine-D_3	432	290
Morphine	429	324, 287

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.1.05 Title: CONFIRMATION - HYDROCODONE IN BLOOD

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

Principle: The deuterium labeled analog of hydrocodone is added to each sample as an internal standard. Hydrocodone and the deuterated internal standard are extracted from blood. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- SPE extraction columns (UCT Clean Screen CSDAU206, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes *
- GC/MS autosampler vials, inserts, and TFE faced caps

* Other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Methanol
- Methylene chloride
- Isopropanol
- Ammonium hydroxide
- Ethyl acetate

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Phosphate buffer, 100 mM, pH 6.0
- Acetate buffer, 100 mM, pH 4.5
- Eluting Solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution - 5 µg/mL Hydrocodone in methanol.
- Internal standard working solution - 2 µg/mL Hydrocodone-D_3 in methanol.

Calibrators and Controls:

Calibrators are prepared in 1 mL aliquots of each of the following 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	Hydrocodone Final Concentration ng/mL	Hydrocodone Working Solution 5 µg/mL	Volume Whole Blood
1	25	5 µL	995 µL
2	50	10 µL	990 µL
3	100	20 µL	980 µL
4	250	50 µL	950 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of 2 µg/mL internal standard to each tube.
3. Add 4 mL of 100 mM phosphate buffer and vortex. Centrifuge for 10 minutes at ~3000 rpm.

Solid Phase Extraction:

4. Place SPE columns into an extraction manifold.
5. Condition SPE columns as follows:
    a. 3 mL methanol
    b. 3 mL distilled water
    c. 1 mL 100 mM phosphate buffer, pH 6.0
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
    a. 2 mL distilled water
    b. 2 mL 100 mM pH 4.5 acetate buffer
    c. 3 mL methanol
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Place labeled 16x100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/isopropanol/ammonium hydroxide (78/20/2, pH=12).
10. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40 ºC under a gentle stream of nitrogen.
11. Add 50 µL of ethyl acetate and vortex.
12. Transfer contents to GC/MS autosampler vials/inserts, and cap. Place samples on an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C mass spectrometer.
Column: HP-5MS, 30m x 0.25 mm i.d., 0.25 µm film thickness or equivalent (i.e. DB-5)
Acquisition method: HYC_ B.M
Data analysis method: HYC_ B.M
GC parameters:

- Injector temp: 250 ºC
- Thermal Aux 2 (Transfer Line): 280 ºC
- MS Source: 230 ºC
- MS Quad: 150 ºC
- Oven program: 100 ºC for 0.25 min, 30 ºC/min to 280 ºC, hold at 280 ºC for 7.25 min.
- Injection mode and volume: splitless, 1-3 µL
- Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Hydrocodone-D_3	302	287
Hydrocodone	299	284, 270

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.1.06 Title: CONFIRMATION - PHENCYCLIDINE IN BLOOD

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

Principle: The deuterium labeled analog of phencyclidine is added to each sample as an internal standard. Phencyclidine and the deuterated internal standard are extracted from blood. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

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

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Ethyl acetate
- Methanol
- Isopropanol
- Ammonium hydroxide

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Acetic acid, 1.0 M
- Phosphate buffer, 100 mM, pH 6.0
- Eluting Solution - ethyl acetate/isopropanol/ammonium hydroxide (90/6/4). Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution – 2.0 µg/mL Phencyclidine-D_5 in methanol.
- Drug working solutions – 1.0 µg/mL Phencyclidine in methanol.

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	Phencyclidine Final Concentration ng/mL	Phencyclidine Working Solution 1 µg/mL	Volume Whole Blood
1	10	10 µL	990 µL
2	15	15 µL	985 µL
3	50	50 µL	950 µL
4	100	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of Phencyclidine-D_5 internal standard (2 µg/mL) to each tube.
3. Add 4 mL of 100 mM Phosphate buffer (pH 6.0) to each tube while vortexing.
4. Centrifuge each tube for 10 min at ~3000 rpm.

Solid Phase Extraction:

5. Place the Cerex Clin II SPE columns into an extraction manifold.
6. Load samples and run through SPE columns.
7. Wash the SPE columns as follows:
   a. 3 mL distilled water
   b. 1 mL 1.0 M acetic acid
   c. 3 mL methanol
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Place collection tubes in manifold under each SPE column and elute with 3 mL of ethyl acetate/isopropanol/ammonium hydroxide (90/6/4).
10. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40 ºC under a gentle stream of nitrogen.
11. Reconstitute in 50 µL ethyl acetate, vortex, and transfer contents of each tube to an autosampler vial/insert/cap and transfer to an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, i.e. DB-5)

Acquisition method: PCP_B.M
Data analysis method: PCP_B.M
GC parameters:

Injector temp: 250 ºC
Thermal Aux 2 (Transfer Line): 300 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 150 ºC for 1 min, 25 ºC/min to 300 ºC, hold at 300 ºC for 3 min
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM) : Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Phencyclidine-D_5	246	190
Phencyclidine	242	200, 243

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.1.07 Title: CONFIRMATION - BENZODIAZEPINES IN BLOOD

Purpose and Scope: This procedure is intended to quantitatively determine alprazolam, clonazepam, diazepam, lorazepam, nordiazepam, oxazepam, temazepam, and triazolam in blood.

Principle: The deuterium labeled analogs of the target analytes are added to each sample as an internal standard. Benzodiazepines and their deuterated internal standards are extracted from blood. The extracted analytes are then converted to the trimethylsilyl derivatives and quantitated by GC/MS operated in SIM mode.

Materials:

- Co-polymer SPE columns, (6 cc 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*
- Autosampler vials, inserts, and TFE faced caps

*other sizes tubes may be used as necessary

Reagents:

Chemicals:

- Ammonium hydroxide
- Ethyl acetate
- Distilled water
- Potassium bicarbonate ($KHCO_3$)
- Potassium carbonate · 1.5-Hydrate ($K_2CO_3 \cdot 1.5H_2O$)
- Glacial acetic acid
- BSTFA with 1% TMCS

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Acetate buffer, 100 mM pH 4.5
- Carbonate buffer
- Eluting Solution – ethyl acetate containing 2% ammonium hydroxide (pH 12).

  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).

  Prepare fresh daily for one time use.

  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution – 2 µg/mL nordiazepam-D_5, oxazepam-D_5, diazepam-D_5, lorazepam-D_4, temazepam-D_5, clonazepam-D_4, alprazolam-D_5, triazolam-D_4 in methanol.
- Drug working solutions – 2 µg/mL nordiazepam, oxazepam, diazepam, lorazepam, temazepam, clonazepam, alprazolam, and triazolam in methanol.

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	Benzodiazepine Mix Final Concentration ng/mL	Benzodiazepine Mix Working Solution 2 µg/mL	Volume Whole Blood
1	25	12.5 µL	987.5 µL
2	50	25 µL	975 µL
3	100	50 µL	950 µL
4	200	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of the deuterated benzodiazepine internal standard (2 µg/mL) to each tube.
3. Add 2.0 mL of 0.1M acetate buffer (pH 4.5) to each tube and vortex.
4. Sonicate the samples for 15 minutes.
5. Centrifuge tubes for 10 minutes 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. 2 mL carbonate buffer
   b. 2 mL distilled water
9. Dry SPE columns for at least 20 minutes at ≥ 20 psi.
10. Place labeled 16 x 100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of ethyl acetate containing 2% ammonium hydroxide (pH=12).

11. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40°C under a gentle stream of nitrogen.
12. Reconstitute with 100 µL ethyl acetate. Add 50 µL of BSTFA w/ 1% TMCS. Cap and incubate at 70 °C for 20 minutes.
13. Allow to cool and transfer the sample from the tube to an autosampler vial/insert, cap and transfer to autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, i.e. DB-5)
Acquisition method: BENZO_BNCI.M
Data analysis method: BENZO_BNCI.M
GC parameters:
Injector temp: 270 ºC
Thermal Aux 2 (Transfer Line): 310 ºC
MS Source: 150 ºC
MS Quad: 106 ºC
Oven program: 150 ºC for 1.0 min, 15 ºC/min to 310 ºC, hold at 310 ºC for 6 min
Injection mode and volume: splitless, 1 - 3 µL
Pressure program: constant flow 1.2 mL/min

MS acquisition parameters (SIM): Use Chemical Ionization Detector (with Negative CI)

Analyte	Quantitation ion	Qualifier ions
Nordiazepam-D_5	239	347
Nordiazepam	234	342,344
Oxazepam-D_5	273	275
Oxazepam	268	270,269
Diazepam-D_5	289	291
Diazepam	284	286,285
Lorazepam-D_4	308	310
Lorazepam	304	303,302
Temazepam-D_5	377	379
Temazepam	372	374,373
Clonazepam-D_4	393	392
Clonazepam	389	388,387
Alprazolam-D_5	313	315
Alprazolam	308	310,309
Triazolam-D_4	312	311
Triazolam	308	307,306

LVMPD FORENSIC LABORATORY TECHNICAL PROCEDURES TOXICOLOGY

4.1.08 Title: CONFIRMATION- CARISOPRODOL / MEPROBAMATE IN BLOOD

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

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*
- Autosampler vials, inserts, and TFE faced caps

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Hexane
- Ethyl acetate

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Acetic acid, 100 mM
- Phosphate buffer, 100 mM, pH 6.0
- Eluting solution - Hexane / Ethyl Acetate (50/50). Prepare fresh daily for one time use.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution – 20 µg/mL of Carisoprodol D_7 (Cerilliant #C-083) and Meprobamate D_7 (Cerilliant #M-131) in methanol.
- Drug working solution**:** 100 µg/mL of Carisoprodol / Meprobamate solution in methanol.

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 ng/mL	Carisoprodol/Meprobamate Working Solution 100 µg/mL	Volume Whole Blood
1	1000	10 µL	990 µL
2	2000	20 µL	980 µL
3	5000	50 µL	950 µL
4	10,000	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework blood specimen into labeled 16 x 125 mm glass culture tubes. Additionally, prepare a 1:10 dilution of each casework blood specimen (pipet 100 µL casework blood specimen and 900 µL of negative whole blood into a labeled 16 x 125 mm glass culture tube).
2. Add 50 µL of (20 µg/mL) internal standard to each tube.
3. Add 4 mL of phosphate buffer (100 mM, pH 6.0) to each tube, vortex.
4. Centrifuge each tube for 10 min at ~3000 rpm.

Solid Phase Extraction:

5. Place the Cerex Clin II SPE columns into an extraction manifold.
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
   a. 3 mL distilled water
   b. 1 mL acetic acid, 100 mM
8. Dry SPE columns for at least 20 minutes at ≥20 psi.
9. Add 2 mL hexane and aspirate.
10. Dry SPE columns for an additional 5 minutes at ≥20 psi.
11. Place labeled 16x100 mm screw top glass culture tubes under each SPE column and elute with 3 mL of hexane/ethyl acetate (50/50).
12. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40°C under a gentle stream of nitrogen.
13. Reconstitute with 100 µL ethyl acetate and vortex.
14. Transfer contents of each tube to an autosampler vial / insert, cap and transfer to autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, i.e. DB-5)

Acquisition method: SOMA_B.M
Data analysis method: SOMA_B.M
GC parameters:
Injector temp: 250 ºC
Thermal Aux 2 (Transfer Line): 280 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 100 ºC for 0.5 min, 30º / min to 280 ºC, hold at 280 ºC for 4.5 min.
Injection mode and volume: split, 20:1 split ratio, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Meprobamate - D_7	151	121
Meprobamate	144	114, 96
Carisoprodol - D_7	191	252
Carisoprodol	245	184, 158

NOTE: A split liner (RESTEK Low Pressure Drop Liner #21033 or equivalent) must be used for this procedure.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.01 Title: CONFIRMATION – AMP / METH / MDA / MDMA IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), and methylenedioxymethamphetamine (MDMA) in urine using a pentafluoropropionic acid anhydride (PFAA) derivative.

Note: PFAA does not convert pseudoephedrine and ephedrine to methamphetamine in appreciable amounts.

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 urine. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- Co-polymer SPE columns, (3cc Cerex® Polycrom™ Clin II 691-0353, or equivalent)
- 16 x 100 mm screw top glass culture tubes with screw caps*
- 12 x 75 mm glass culture tubes*
- 10 x 75 mm glass culture tubes*
- 10 mm snap-caps
- GC/MS autosampler vials, inserts, and TFE faced caps

* Other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Methanol
- Ethyl acetate
- Ammonium hydroxide
- Pentafluoropropionic acid anhydride (PFAA)

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- 1% Acidic methanol
- Phosphate buffer, 100 mM, pH 6.0
- Acetic acid, 100 mM
- Eluting Solution – ethyl acetate containing 2% ammonium hydroxide (pH 12).
  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.

QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution – 20 µg/mL of Amp-D_{11} / Meth-D_{14} / MDA-D_5 / MDMA-D_5 in methanol.
- Drug working solution – 10 µg/mL of Amp/Meth/MDA / MDMA in methanol.

Calibrators and Controls:

Calibrators are prepared in 1 mL aliquots of each of the concentrations listed below in labeled 12 x 75 mm glass culture tubes using negative urine 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.

NOTE: A commercially prepared control (i.e. Biochemical Diagnostics Inc. Detectabuse® # 18001225) may be used as the control prior to case samples.

Calibrator / Control	Final Concentration ng/mL	Amp/Meth/MDA/MDMA Working Solution 10 µg/mL	Volume Negative Urine
1	100	10 µL	990 µL
2	250	25 µL	975 µL
3	500	50 µL	950 µL
4	1000	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework urine specimen and the external urine control, if applicable, into labeled 12 x 75 mm glass culture tubes.
2. Add 25 µL of (20 µg/mL) internal standard mix to each tube.
3. Add 1 mL of 100 mM Phosphate Buffer (pH 6.0) to each tube.

Solid Phase Extraction:

4. Place the Cerex Polycrom Clin II SPE columns into an extraction manifold.
5. Load samples and run through SPE columns.
6. Wash the SPE columns as follows:
   a. 1 mL distilled water
   b. 1 mL 100 mM acetic acid
   c. 1 mL methanol
   d. 1 mL ethyl acetate

7. Dry SPE columns for at least 10 minutes at ≥20 psi under a gentle steam of nitrogen.
8. Place labeled 10 x 75 mm culture tubes under each SPE column and elute with 1 mL of ethyl acetate containing 2% ammonium hydroxide (pH=12).
9. Add 100 µL of 1% acidic methanol to each tube.
10. Transfer the tubes to an evaporator bath and evaporate to dryness at 30 °C under a gentle stream of nitrogen. **Do not over dry because the amphetamine compounds are volatile.**

Derivatization:

11. Add 50 µL of PFAA and 50 µL of ethyl acetate to each tube and cap. Incubate at 70 C for 30 minutes. Remove from heat and allow the samples to cool.
12. Transfer tubes to the evaporator bath and again evaporate to dryness at 30 °C under a gentle stream of nitrogen.
13. Reconstitute with 50 µL of ethyl acetate. Transfer contents to GC/MS autosampler vials/inserts, cap and transfer samples to an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, i.e. DB-5)
Acquisition method: METH_U.M
Data analysis method: METH_U.M
GC parameters:

- Injector temp: 250 C
- Thermal Aux 2 (Transfer Line): 280 C
- MS Source: 230 C
- MS Quad: 150 C
- Oven program: 70 C for 1 min, 20 C/min to 280 C, hold at 280 C for 3 min.
- Injection mode and volume: splitless, 1-3 µL
- Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Amphetamine-D_{11}	194	128
Amphetamine	190	118, 91
Methamphetamine-D_{14}	211	163
Methamphetamine	204	160, 118
MDA-D_5	330	167
MDA	325	190, 162
MDMA-D_5	208	344
MDMA	204	339, 160

Special Reporting Criteria:

To be reported positive for methamphetamine a specimen must also contain amphetamine at a concentration equal or greater than 100 ng/mL.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.02 Title: CONFIRMATION - COCAINE / BENZOYLECGONINE IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine cocaine and benzoylecgonine (cocaine metabolite) in urine.

Principle: The deuterium labeled analog of cocaine and benzoylecgonine is added to each sample as an internal standard. Cocaine, benzoylecgonine and the deuterated internal standards are extracted from urine. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- SPE columns (UCT Clean Screen ® CSDAU206, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes with screw caps*
- Autosampler vials, inserts, and TFE faced caps*

*other sizes tubes may be used as necessary

Reagents:

Chemicals:

- Methanol
- Distilled water
- Ethyl acetate
- Ammonium hydroxide
- BSTFA with 1% TMCS

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Hydrochloric acid, 0.1 M
- Phosphate buffer, 100 mM, pH 6.0
- Eluting solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as necessary to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution – 10 µg/mL of Cocaine / BZE in methanol.
- Internal standard working solution - 10 µg/mL Cocaine-D_3 / BZE-D_3 in methanol.

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 urine 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 ng/mL	COC/BZE Working Solution 10 µg/mL	Volume Negative Urine
1	100	10 µL	990 µL
2	150	15 µL	985 µL
3	500	50 µL	950 µL
4	1000	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework urine specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of (10 µg/mL) internal standard solution to each tube.
3. Add 2 mL of 100 mM phosphate buffer (pH 6.0) to each tube. Vortex.

Solid Phase Extraction:

4. Place SPE extraction columns into an extraction manifold.
5. Condition SPE columns as follows:
   a. 3 mL methanol
   b. 3 mL distilled water
   c. 1 mL 100 mM phosphate buffer (pH 6.0)
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
   a. 3 mL distilled water
   b. 2 mL 0.1 M hydrochloric acid
   c. 3 mL methanol
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Place 16 x 100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/ isopropanol/ ammonium hydroxide solution (78/20/2, pH = 12).
10. Transfer tubes to an evaporator bath and evaporate to dryness at 40 °C under a gentle stream of nitrogen.

Derivatization:

11. Add 50 µL of ethyl acetate and 50 µL of BSTFA with 1% TMCS to each tube and cap. Incubate at 70 °C for 20 minutes. Remove from heat and allow the samples to cool.
12. Transfer contents to GC/MS autosampler vials/inserts, cap and transfer samples to an autosampler tray for GC/MS analysis.

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GC/MS Analysis:

Instrumentation: Agilent Technologies 7890A GC and 5975C Mass Spectrometer.

Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent)
Acquisition method: COC_U.M
Data analysis method: COC_U.M
GC parameters:

Injector Temp: 250 C
Thermal Aux 2 (Transfer Line): 300 C
MS Source: 230 C
MS Quad: 150 C
Oven program: 100 C for 0.25 min, 35 C/min to 220 C, 20 C/min to 300 C, hold at 300 C for 4 min.
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters(SIM) : Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Cocaine-D_3	185	201
Cocaine	182	198, 303
Benzoylecgonine-D_3	243	259
Benzoylecgonine	240	256, 361

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TECHNICAL PROCEDURES
TOXICOLOGY

4.2.03 Title: CONFIRMATION - THCA IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine in urine the principle metabolite of marijuana, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (also called THCA, THC-COOH, carboxy THC, 11-COOH-THC, or THC carboxylic acid).

Principle: The deuterium labeled analog of THCA is added to each sample as an internal standard. THCA and the deuterated internal standard are extracted from urine and analyzed by LC/MS/MS.

Materials:

- SPE column (UCT Clean Screen ® CSTHC206, or equivalent)
- 16 x 100 mm screw top silanized glass culture tubes with screw caps (other size tubes may be used as necessary)
- 16 x 100 mm silanized glass culture tubes (other size tubes may be used as necessary)
- Autosampler vials and caps

Reagents:

Chemicals:

- Acetonitrile
- Distilled water
- Ethyl acetate
- Glacial acetic acid
- Hexane

Methanol Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Sodium hydroxide 10 N
- Hydrochloric acid, 100 mM
- 100 mM hydrochloric acid / acetonitrile (60/40). Prepare fresh daily for one time use.
- Eluting solution - hexane / ethyl acetate / glacial acetic acid (80/18/2). Prepare fresh daily for one time use.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution - THCA-D_3, 1 µg/mL in methanol.
- Drug standard working solution – THCA 1.5 µg/mL in methanol.
- Drug control working solution – THCA 1.5 µg/mL in methanol.
- THCA glucuronide control working solution – THCA glucuronide 1 µg/mL 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 screw top silanized glass culture tubes using negative urine 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.

A THCA glucuronide control will be used to verify the effectiveness of the hydrolysis step and is run following the negative control.

NOTE: A commercially prepared control (i.e. Biochemical Diagnostics Inc. Detectabuse® # 18001225) may be used as the control prior to case samples.

Calibrator / Control	THCA Final Concentration ng/mL	THCA Working Solution 1.5 µg/mL	Volume Negative Urine
1	15	10 µL	990 µL
2	30	20 µL	980 µL
3	75	50 µL	950 µL
4	150	100 µL	900 µL
5	300	200 µL	800 µL
Negative Control	0	0	1000 µL

Control	THCA Final Concentration ng/mL	THCA Glucuronide Control Working Solution 1 µg/mL	Volume Negative Urine
THCA Glucuronide Control	66	100 µL	900 µL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework urine specimen, and the commercially prepared urine control as applicable, into labeled 16 x 100 mm screw top silanized glass culture tubes.
2. Add 50 µL of (1 µg /mL) internal standard and 50 µL of 10 N sodium hydroxide, to each tube, cap and vortex.
3. Hydrolyze for 20 min at 60 ºC in an oven/heat block. Remove from the heat source and allow to cool.
4. Add 500 µL of glacial acetic acid and vortex.
   Note: The addition of glacial acetic acid adjusts the pH of the urine to approximately 3.5 ± 0.5. If THCA/THCA-D_3 is not extracted or poorly extracted from a urine sample, the pH should be checked to ensure it is approximately 3.5 ± 0.5 when the sample is re-extracted.

Solid Phase Extraction:

5. Place SPE columns into an extraction manifold and condition as follows:
    a. 3 mL methanol
    b. 3 mL distilled water
    c. 1 mL 100 mM hydrochloric acid
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
    a. 2 mL distilled water
    b. 2 mL (60/40) 100 mM hydrochloric acid / acetonitrile
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Add 200 µL hexane and dry SPE columns for at least 5 minutes at ≥ 20 psi.
10. Place labeled 16x100 mm silanized glass culture tubes in manifold under each SPE column and elute with 3 mL of (80/18/2) hexane / ethyl acetate / glacial acetic acid.
11. Transfer the tubes to an evaporator bath and evaporate to dryness at 40° C under a gentle stream of nitrogen.

Reconstitution:

12. Add 500 µL methanol to each tube and vortex
13. Add 500 µL distilled water to each tube and vortex.
14. Transfer the contents of each tube into an LC/MS autosampler vial. Cap and transfer to autosampler tray for LC/MS analysis.

LC/MS Analysis:

LVMPD Instrument: Tox #1 LC/MS
Instrument Make/Model: Agilent 6420 LC/MS/MS
Acquisition Method: THCA_U.M
Data Analysis Method: THCA_U.M

LC Parameters:

Multisampler Temperature	4.0 ºC – Room Temperature	
Injection Volume	2.0 – 30.0 µL	
Column	Agilent Poroshell 120 EC-C18 (3.0 x 50 mm, 2.7 µm)	
Column Temperature	40 ºC	
Needle Wash	10 s	
Needle Wash Solution	1:1:1:1 Methanol:Water:Acetonitrile:2-Propanol	
Mobile Phase A	5 mM Ammonium Acetate in Water	
Mobile Phase B	Acetonitrile	
Flow Rate	0.4 mL/min	
Gradient	Initial	40% B
	1.0 minutes	40% B
	7.0 minutes	95% B
Stop Time	10.5 minutes	
Post Time	3.0 minutes	

MSD Parameters

Parameter	Value
Ionization	ESI
Polarity	Positive
Gas Temperature	350 ºC
Gas Flow	10 L/min
Nebulizer Pressure	20 psi
Capillary	5,000 V

Analyte	Quantitation Transition	Qualifier Transition
THCA-D3	348.2 → 302.1	n/a
THCA	345.2 → 299.1	345.2 → 192.9

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.04 Title: CONFIRMATION - CODEINE / MORPHINE IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine total codeine and total morphine in urine. When morphine is present above the reporting threshold, continue with confirmation of 6-acetylmorphine (Chapter 4.2.08).

Principle: The deuterium labeled analog of codeine and morphine is added to each sample as an internal standard. Codeine, morphine and the deuterated internal standards are extracted from urine. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- SPE columns (UCT Clean Screen CSDAU206, or equivalent)
- 16 x 100 mm screw top glass culture tubes *
- GC/MS autosampler vials, inserts, and TFE faced caps*

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Methanol
- BSTFA with 1% TMCS
- Ethyl acetate

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Red abalone β-glucuronidase enzyme solution in 100 mM acetate buffer pH 5.0 (5,000 units/mL, e.g. UCT BETA-GLUC-10 or equivalent). Prepare fresh daily for one time use.
- Acetate buffer, 100 mM, pH 5.0
- Phosphate buffer, 100 mM, pH 6.0
- Acetate buffer, 100 mM, pH 4.5
- Eluting Solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12) Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution - 50 µg/mL COD/MOR in methanol.
- Internal standard working solution - 20 µg/mL COD-D_3 / MOR-D_3 in methanol.

- Morphine-3β-D-Glucuronide control working solution - Morphine-3β-D-Glucuronide 3240 ng/mL in negative urine

Calibrators and Controls:

Calibrators are prepared in 1 mL aliquots of each of the concentrations listed below in labeled 16 x 100 mm screw top glass culture tubes using negative urine 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.

A Morphine-3β-D-Glucuronide control will be used to verify the effectiveness of the hydrolysis step and is run following the negative control. This control may be prepared in-house or commercially prepared (e.g., Biochemical Diagnostics Inc. Detectabuse® # 18001225).

NOTE: A commercially prepared control (e.g., Biochemical Diagnostics Inc. Detectabuse® # 18001225) may be used as the control prior to case samples.

Calibrator / Control	Codeine / Morphine Final Concentration ng/mL	Codeine / Morphine Working Solution 50 µg/mL	Volume Negative Urine
1	1000	20 µL	980 µL
2	2000	40 µL	960 µL
3	4000	80 µL	920 µL
4	5000	100 µL	900 µL
Negative Control	0	0	1 mL

Control	Total Morphine Final Concentration ng/mL	Morphine-3β-D-Glucuronide Control Working Solution 3240 ng/mL	Volume Negative Urine
Morphine-3β-D-Glucuronide Control	2000	1 mL	0

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework urine specimen into labeled 16 x 100 mm screw top glass culture tubes.
2. Add 50 µL of 20 µg/mL internal standard to each tube.
3. Add 1 mL prepared red abalone β-glucuronidase enzyme solution in acetate buffer. Vortex. Incubate for 90 minutes at 65 ºC. Cool before proceeding.
4. Add 2 mL of 100 mM pH 6.0 phosphate buffer. Mix / vortex.

Solid Phase Extraction:

5. Place SPE columns into an extraction manifold.
6. Condition SPE columns as follows:
    a. 3 mL methanol
    b. 3 mL distilled water
    c. 1 mL 100 mM phosphate buffer, pH 6.0
7. Load samples and run through SPE columns.
8. Wash SPE columns as follows:
    a. 2 mL distilled water
    b. 2 mL 100 mM pH 4.5 acetate buffer
    c. 3 mL methanol
9. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
10. Place labeled 16x100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/isopropanol/ammonium hydroxide **(78/20/2)**.
11. Transfer collection tubes to an evaporator bath and evaporate to dryness at ~40ºC under a gentle stream of nitrogen.

Derivatization:

12. Add 50 µL of BSTFA and 50 µL of ethyl acetate to each tube, cap and incubate at ~ 70 ºC for 30 minutes. Allow to cool.
13. Transfer sample from each tube to an autosampler vial/insert for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C mass spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent e.g., DB-5)
Acquisition method: OPI_U.M
Data analysis method: OPI_U.M
GC parameters:
Injector temp: 250 ºC
Thermal Aux 2 (Transfer Line): 280 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 100 ºC for 0.25 min, 30 ºC/min to 280 ºC hold at 280 ºC for 7.25 min.
Injection mode and volume: split, 10:1 split ratio, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Codeine-D_3	374	346
Codeine	371	343, 234
Morphine-D_3	432	290
Morphine	429	324, 287

NOTE: A split liner (RESTEK Low Pressure Drop Liner #21033 or equivalent must be used for this procedure.

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.05 Title: CONFIRMATION - HYDROCODONE IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine hydrocodone in urine.

Principle: The deuterium labeled analog of hydrocodone is added to each sample as an internal standard. Hydrocodone and the deuterated internal standard are extracted from urine. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- SPE columns (UCT Clean Screen CSDAU206, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes *
- GC/MS autosampler vials, inserts, and TFE faced caps

Other size tubes may be used as necessary

Reagents:

Chemicals

- Distilled water
- Methanol
- Methylene chloride
- Isopropanol
- Ammonium hydroxide
- Ethyl acetate

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Phosphate buffer, 100 mM, pH 6.0
- Acetate buffer, 100 mM, pH 4.5
- Eluting Solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution - 5 µg/mL Hydrocodone in methanol.
- Internal standard working solution - 2 µg/mL Hydrocodone-D_3 in methanol.

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 urine 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	Hydrocodone Final Concentration ng/mL	Hydrocodone Working Solution 5 µg/mL	Volume Negative Urine
1	100	20 µL	980 µL
2	200	40 µL	960 µL
3	400	80 µL	920 µL
4	500	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework urine specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 50 µL of 2 µg/mL internal standard to each tube.
3. Add 2 mL of 100 mM phosphate buffer and vortex.

Solid Phase Extraction:

4. Place SPE columns into an extraction manifold.
5. Condition SPE columns as follows:
    a. 3 mL methanol
    b. 3 mL distilled water
    c. 1 mL 100 mM phosphate buffer, pH 6.0
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
    a. 2 mL distilled water
    b. 2 mL 100 mM pH 4.5 acetate buffer
    c. 3 mL methanol
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Place labeled 16x100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/isopropanol/ammonium hydroxide (78/20/2, pH=12).
10. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40 ºC under a gentle stream of nitrogen.
11. Add 100 µL of ethyl acetate and vortex.
12. Transfer contents to GC/MS autosampler vials/inserts, and cap. Place samples on an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C mass spectrometer.
Column: HP-5MS, 30m x 0.25 mm i.d., 0.25 µm film thickness or equivalent (e.g. DB-5)
Acquisition method: HYC_ U.M
Data analysis method: HYC_ U.M
GC parameters:

Injector temp: 280 ºC
Thermal Aux 2 (Transfer Line): 280 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 100 ºC for 0.25 min, 30 ºC/min to 280 ºC, hold at 280 ºC for 7.25 min.
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Hydrocodone-D_3	302	287
Hydrocodone	299	284, 270

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.06 Title: CONFIRMATION - PHENCYCLIDINE IN URINE

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

Principle: The deuterium labeled analog of phencyclidine is added to each sample as an internal standard. Phencyclidine and the deuterated internal standard are extracted from urine. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- Co-polymer SPE columns, (3 cc Cerex® Polycrom™ Clin II 691-0353, or equivalent)
- 12 x 75 mm glass culture tubes*
- 10 x 75 mm glass culture tubes*
- Autosampler vials, inserts, and TFE faced caps

*other size tubes may be used as necessary

Reagents:

Chemicals:

- Ethyl acetate
- Methanol
- Isopropanol
- Ammonium hydroxide

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Phosphate buffer, 100 mM, pH 6.0
- Acetic acid, 0.1 M
- Eluting Solution - ethyl acetate/isopropanol/ammonium hydroxide (90/6/4). Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution – 2.0 µg/mL Phencyclidine-D_5 in methanol.
- Drug working solutions – 1.0 µg/mL Phencyclidine in methanol.

Calibrators and Controls:

Calibrators are prepared in 1 mL aliquots of each of the concentrations listed below in labeled 12 x 75 mm glass culture tubes using negative urine 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.

NOTE: A commercially prepared control (e.g., Biochemical Diagnostics Inc. Detectabuse® # 18001225) may be used as the control prior to case samples.

Calibrator / Control	Phencyclidine Final Concentration ng/mL	Phencyclidine Working Solution 1 µg/mL	Volume Negative Urine
1	15	15 µL	985 µL
2	25	25 µL	975 µL
3	50	50 µL	950 µL
4	100	100 µL	900 µL
Negative Control	0	0	1 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 1 mL of each casework urine specimen into labeled 12 x 75 mm glass culture tubes.
2. Add 25 µL of Phencyclidine-D_5 internal standard (2 µg/mL) to each tube.
3. Add 1 mL of 100 mM Phosphate buffer (pH 6.0) to each tube and vortex.

Solid Phase Extraction:

4. Place the Cerex Polycrom Clin II SPE columns into an extraction manifold.
5. Load samples and run through SPE columns.
6. Wash the SPE columns as follows:
    a. 1 mL distilled water
    b. 1 mL 0.1 M acetic acid
    c. 1 mL methanol
7. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
8. Place collection tubes in manifold under each SPE column and elute with 2 mL of ethyl acetate/isopropanol/ammonium hydroxide (90/6/4).
9. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40ºC under a gentle stream of nitrogen.
10. Reconstitute in 50 µL ethyl acetate, vortex, and transfer contents of each tube to an autosampler vial/insert/cap and transfer to an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, e.g. DB-5)
Acquisition method: PCP_U.M
Data analysis method: PCP_U.M
GC parameters:

Injector temp: 250 ºC
Thermal Aux 2 (Transfer Line): 300 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 150 ºC for 1 min, 25 ºC/min to 300 ºC, hold at 300 ºC for 3 min
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM) : Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Phencyclidine-D_5	246	190
Phencyclidine	242	200, 186

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.07 Title: CONFIRMATION - BENZODIAZEPINES IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine in urine nordiazepam, oxazepam, lorazepam, temazepam, α-OH-alprazolam, and α-OH-triazolam.

Principle: The deuterium labeled analogs of the target analytes are added to each sample as an internal standard. Benzodiazepines and their deuterated internal standards are extracted from urine. The extracted analytes are then converted to the trimethylsilyl derivitives and quantitated by GC/MS operated in SIM mode.

Materials:

- SPE columns (UCT Clean Screen CSDAU206, or equivalent)
- 16 x 100 mm screw top glass culture tubes *
- GC/MS autosampler vials, inserts, and TFE faced caps

*Other size tubes may be used as necessary

Reagents:

Chemicals:

- Distilled water
- Sodium phosphate dibasic, anhydrous (Na_2HPO_4)
- Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$)
- Acetonitrile
- Hexane
- Ethyl acetate
- BSTFA with 1% TMCS

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- β-glucuronidase enzyme solution (5000 units/mL) in 100 mM acetate buffer pH 5.0. Prepare fresh daily for one time use.
- Phosphate buffer, 100 mM, pH 6.0
- Acetate buffer, 100 mM, pH 5.0

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Internal standard working solution - 10 µg/mL each of nordiazepam-D_5, oxazepam-D_5, lorazepam-D_4, temazepam-D5, α-OH-alprazolam-D_5 and α-OH-triazolam-D_4 in methanol.
- Drug working solutions – 10 µg/mL each of nordiazepam, oxazepam, lorazepam, temazepam, α-OH-alprazolam, and α-OH-triazolam in methanol.
- Oxazepam glucuronide control working solution – 10 µg/mL of oxazepam glucuronide in distilled water.

Calibrators and Controls:

Calibrators are prepared in 2 mL aliquots of each of the following concentrations listed below in labeled 12 x 75 mm glass culture tubes using negative urine 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.

An oxazepam glucuronide control will be used to verify the effectiveness of the hydrolysis step and is run immediately following the negative control.

NOTE: A commercially prepared control (i.e. Biochemical Diagnostics Inc. Detectabuse® # 18002165) may be used as the control prior to case samples.

Calibrator / Control	Benzodiazepine Mix Final Concentration ng/mL	Benzodiazepine Mix Working Solution 10 µg/mL	Volume Negative Urine
1	50	10 µL	1.990 mL
2	100	20 µL	1.980 mL
3	250	50 µL	1.950 mL
4	500	100 µL	1.900 mL
Negative Control	0	0	2 mL

Control	Oxazepam Final Concentration ng/mL	Oxazepam Glucuronide Control Working Solution 10 µg/mL	Volume Negative Urine
Oxazepam Glucuronide Control	310	100 µL	1.900 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 2 mL of each casework urine specimen into labeled 16 x 100 mm glass culture tubes.
2. Add 50 µL of the deuterated benzodiazepine internal standard (10 µg/mL) to each tube.
3. Add 1mL prepared β-glucuronidase enzyme solution in acetate buffer. Vortex. Incubate for 3 hours at 65 ºC or overnight at 37 ºC. Cool before proceeding.

Solid Phase Extraction:

4. Place SPE columns into an extraction manifold.
   Condition SPE columns as follows:
   a. 3 mL methanol
   b. 3 mL distilled water

c. 1 mL phosphate buffer, 100 mM (pH 6.0)

5. Load samples and run through SPE columns.
6. Wash SPE columns as follows:
   a. 2 mL distilled water
   b. 2 mL 20% acetonitrile in phosphate buffer, 100 mM (pH 6.0)
7. Dry SPE columns for at least 10 minutes at ≥ 20 psi.
8. Add 2 mL hexane to each column, aspirate and let dry for another 5 minutes.
9. Place labeled 16 x 100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of ethyl acetate.
10. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40°C under a gentle stream of nitrogen.

Derivatization:

11. Add 50 µL BSTFA with 1% TMCS, 50 uL ethyl acetate and cap. Incubate at 70°C for 20 minutes. Allow to cool.
12. Transfer contents of each tube to an autosampler vial/insert for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C Mass Spectrometer.
Column: HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, i.e. DB-5)
Acquisition method: BENZO_U.M
Data analysis method: BENZO_U.M
GC parameters:
Injector temp: 270 ºC
Thermal Aux 2 (Transfer Line): 290 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 150 ºC for 1.0 min, 30 ºC /min to 290 ºC, hold at 290 ºC for 12 min
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters: (SIM) Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
Nordiazepam-D_5	347	346
Nordiazepam	341	342,327
Oxazepam-D_5	433	406
Oxazepam	429	430, 401
Lorazepam-D_4	434	433
Lorazepam	429	430, 431
Temazepam-D_5	348	350
Temazepam	343	283, 257
α-OH-alprazolam-D_5	386	388
α-OH-alprazolam	381	396,383
α-OH-triazolam-D_4	421	436
α-OH-triazolam	415	430, 380

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.2.08 Title: CONFIRMATION - 6-ACETYLMORPHINE IN URINE

Purpose and Scope: This procedure is intended to quantitatively determine 6-acetyl-morphine (6-AM) in urine.

Principle: The deuterium labeled analog of 6-AM is added to each sample as an internal standard. 6-AM and the deuterated internal standard are extracted from urine. The extracted analytes are then quantitated by GC/MS operated in SIM mode.

Materials:

- SPE columns (UCT Clean Screen CSDAU206, or equivalent)
- 16 x 125 mm glass culture tubes*
- 16 x 100 mm screw top glass culture tubes *
- GC/MS autosampler vials, inserts, and TFE faced caps

* Other size tubes may be used as necessary

Reagents:

Chemicals

- Distilled water
- Methanol
- Methylene chloride
- Isopropanol
- Ammonium hydroxide
- Ethyl acetate
- BSTFA with 1% TMCS

Reagent Solutions (Unless specified below, see Chapter 4.4 for preparation, QC, and storage instructions):

- Phosphate buffer, 100 mM, pH 6.0
- Acetate buffer, 100 mM, pH 4.5
- Eluting Solution - methylene chloride/isopropanol/ammonium hydroxide (78/20/2).
  Add additional ammonium hydroxide as needed to achieve the required pH (pH = 12).
  Prepare fresh daily for one time use.
  QC: Check pH with pH paper.

Drug Solutions (see Chapter 4.3 for preparation and storage instructions. See Section 6.4 for QC instructions):

- Drug working solution - 2 µg/mL 6-AM in acetonitrile.
- Internal standard working solution – 1 µg/mL 6-AM-D_3 in acetonitrile.

Calibrators and Controls:

Calibrators are prepared in 2 mL aliquots of each of the concentrations listed below in labeled 16 x 125 mm glass culture tubes using negative urine 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	6-AM Final Concentration ng/mL	6-AM Working Solution 2 µg/mL	Volume Negative Urine
1	10	10 µL	1.990 mL
2	20	20 µL	1.980 mL
3	50	50 µL	1.950 mL
4	100	100 µL	1.900 mL
Negative Control	0	0	2 mL

Preparation:

1. Prepare calibrators and controls as described above. Pipet 2 mL of each casework urine specimen into labeled 16 x 125 mm glass culture tubes.
2. Add 25 µL of 1 µg/mL internal standard to each tube.
3. Add 1 mL of 100 mM phosphate buffer and vortex.

Solid Phase Extraction:

4. Place SPE columns into an extraction manifold.
5. Condition SPE columns as follows:
    a. 3 mL methanol
    b. 3 mL distilled water
    c. 1 mL 100 mM phosphate buffer, pH 6.0
6. Load samples and run through SPE columns.
7. Wash SPE columns as follows:
    a. 2 mL distilled water
    b. 2 mL 100 mM pH 4.5 acetate buffer
    c. 3 mL methanol
8. Dry SPE columns for at least 15 minutes at ≥ 20 psi.
9. Place labeled 16x100 mm screw top glass culture tubes in manifold under each SPE column and elute with 3 mL of methylene chloride/isopropanol/ammonium hydroxide (78/20/2, pH=12).
10. Transfer collection tubes to an evaporator bath and evaporate to dryness at 40 ºC under a gentle stream of nitrogen.

Derivatization:

11. Add 50 µL of ethyl acetate and 50 µL of BSTFA with 1% TMCS to each tube and cap. Incubate at ~70 ºC for 20 min. Remove from heat and allow the samples to cool.
12. Transfer contents to GC/MS autosampler vials/inserts, cap and transfer samples to an autosampler tray for GC/MS analysis.

GC/MS Analysis:

Instrumentation: Agilent 7890A GC and 5975C mass spectrometer.
Column: HP-5MS, 30m x 0.25 mm i.d., 0.25 µm film thickness (or equivalent, e.g., DB-5)
Acquisition method: 6-AM_ U.M
Data analysis method: 6-AM_ U.M
GC parameters:

Injector temp: 250 ºC
Thermal Aux 2 (Transfer Line): 290 ºC
MS Source: 230 ºC
MS Quad: 150 ºC
Oven program: 150 ºC for 0.25 min, 35 ºC/min to 290 ºC, hold at 290 ºC for 5.75 min.
Injection mode and volume: splitless, 1-3 µL
Pressure program: constant flow 1 mL/min

MS acquisition parameters (SIM): Use Electron Impact Detector

Analyte	Quantitation ion	Qualifier ions
6-MAM-D_3	402	290
6-MAM	399	340,287

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

4.3 Title: CONFIRMATION – DRUG SOLUTION PREPARATIONS

Note: Variations to the formulations must be approved by the Forensic Toxicology Manager, or designee. Approval is indicated on the Reagent Prep form.

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 date of a component of the preparation, whichever is sooner.

Solvents:
Methanol and acetonitrile used for preparations should be GC grade or better.

Quality Control:
See section **6.4 Quality Control Checks of Drug Stock and Working Solutions** for Quality Control procedures.

AMP / METH / MDA / MDMA Blood and Urine Stock and Working Solutions

Note: The LVMPD Forensic Laboratory does not distinguish between +/- AMP, +/- METH, +/- MDA and +/- MDMA when reporting drug confirmation results

100 µg/mL AMP calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL AMP Cerilliant standard (A-007)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL METH calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL METH Cerilliant standard (M-009)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL MDA calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL MDA Cerilliant standard (M-012)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL MDMA calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL MDMA Cerilliant standard (M-013)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL AMP / METH / MDA / MDMA calibration standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL AMP calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL METH calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL

1 mL	100 µg/mL MDA calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL MDMA calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL AMP / METH / MDA / MDMA control working solution

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

100 µg/mL AMP-D_{11} internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL AMP-D_{11} Cerilliant standard (A-019)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL METH-D_{14} internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL METH-D_{14} Cerilliant standard (M-093)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL MDA-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL MDA-D_5 Cerilliant standard (M-027)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL MDMA-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL MDMA-D_5 Cerilliant standard (M-029)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

20 µg/mL AMP-D_{11} / METH-D_{14} / MDA-D_5 / MDMA-D_5 internal standard working solution

Amount	Ingredients	Measuring Device
2 mL	100 µg/mL AMP-D_{11} internal standard stock solution	1 mL Class A micro volumetric flask or pipette(s) suitable for measuring 2 mL
2 mL	100 µg/mL METH-D_{14} internal standard stock solution	1 mL Class A micro volumetric flask or pipette(s) suitable for measuring 2 mL
2 mL	100 µg/mL MDA-D_5 internal standard stock solution	1 mL Class A micro volumetric flask or pipette(s) suitable for measuring 2 mL
2 mL	100 µg/mL MDMA-D_5 internal standard stock solution	1 mL Class A micro volumetric flask or pipette(s) suitable for measuring 2 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

COC / BZE Blood and Urine Stock and Working Solutions

100 µg/mL COC calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1 mL of a 1.0 mg/mL COC Cerilliant standard (C-008))	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Acetonitrile	10 mL Class A volumetric flask

100 µg/mL BZE calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL BZE Cerilliant standard (B-004)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL COC / BZE calibration standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL COC calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL BZE calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL COC / BZE control working solution

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

10 µg/mL COC-D3 / BZE-D3 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL COC-D_3 Cerilliant standard (C-004)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL BZE-D_3 Cerilliant standard (B-001)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

THC / THCA Blood and Urine 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 (-)-Δ9-THC Cerilliant standard (T-005)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL THC calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL THC calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL 11-OH-THC calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL (±)-11-OH-Δ9-THC Cerilliant standard (H-026)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol (*GC grade or better*)	10 mL Class A volumetric flask

10 µg/mL THCA calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL (-)-11-nor-9-Carboxy- Δ9-THC Cerilliant standard (T-018)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1.5 µg/mL THCA calibration standard working solution

Amount	Ingredients	Measuring Device
1.5 mL	10 µg/mL (-)-11-nor-9-Carboxy- Δ9-THC Cerilliant standard (T-018)	Pipette(s) suitable for measuring 1.5 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1.5 µg/mL THCA control working solution

Prepare a 1.5 µg/mL THCA control working solution using a similar formulation as described for preparing the 1.5 µg/mL THCA calibration standard working solution, with the exception that the control material must come from a different manufacturer than Cerilliant. A 100 µg/mL control stock solution is required if the material comes in a 1.0 mg/mL THCA concentration.

0.1 µg/mL THC, 11-OH-THC / 0.5 µg/mL THCA calibration standard working solution – Level 1

Amount	Ingredients	Measuring Device
1 mL	1 µg/mL THC, 11-OH-THC / 5 µg/mL THCA calibration standard working solution – Level 2	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1 µg/mL THC, 11-OH-THC / 5 µg/mL THCA calibration standard working solution – Level 2

Amount	Ingredients	Measuring Device
1 mL	10 µg/mL THC standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	10 µg/mL 11-OH-THC standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
500 µL	100 µg/mL (-)-11-nor-9-Carboxy- Δ9-THC Cerilliant standard (T-018)	Pipette suitable for measuring 500 µL
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.

10 µg/mL THC-D_3 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL (-)-Δ9-THC-D_3 Cerilliant standard (T-003)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL 11-OH-THC-D_3 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL 11-OH- Δ9-THC-D_3 Cerilliant standard (H-041)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL THCA-D_3 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL (±)-11-nor-9-Carboxy-Δ9-THC-D_3 Cerilliant standard (T-004)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1 µg/mL THCA-D_3 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	10 µg/mL (±)-11-nor-9-Carboxy-Δ9-THC-D_3 Cerilliant standard (T-004)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

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
500 µL	10 µg/mL THC-D_3 internal standard stock solution	A pipette suitable for measuring 500 µL
500 µL	10 µg/mL 11-OH-THC-D_3 internal standard stock solution	A pipette suitable for measuring 500 µL
2.5 mL	10 µg/mL THCA-D_3 internal standard stock solution	A pipette suitable for measuring 2.5 mL
QS to 50 mL	Methanol	50 mL Class A volumetric flask

10 µg/mL THCA Glucuronide control stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL THCA Glucuronide Cerilliant standard (T-038)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1 µg/mL THCA Glucuronide control working solution

Amount	Ingredients	Measuring Device
1 mL	10 µg/mL THCA Glucuronide control stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

Codeine / Morphine Blood and Urine Stock and Working Solutions

100 µg/mL Codeine calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Codeine Cerilliant standard (C-006)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Morphine calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Morphine Cerilliant standard (M-005)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL Codeine / Morphine calibration standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Codeine calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Morphine calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL Codeine / Morphine control working solution

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

50 µg/mL Codeine / Morphine calibration standard working solution

Amount	Ingredients	Measuring Device
500 µL	1.0 mg/mL Codeine Cerilliant standard (C-006)	Pipette suitable for measuring 500 µL
500 µL	1.0 mg/mL Morphine Cerilliant standard (M-005)	Pipette suitable for measuring 500 µL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

50 µg/mL Codeine / Morphine control working solution

Prepare a 50 µg/mL Codeine / Morphine control working solution using a similar formulation as described for preparing the 50 µg/mL Codeine / Morphine calibration standard working solution, with the exception that the control material must come from a different manufacturer than Cerilliant.

20 µg/mL Codeine-D_3 / Morphine-D_3 internal standard stock/working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Codeine-D_3 Cerilliant standard (C-005)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Morphine-D_3 Cerilliant standard (M-003)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

2 µg/mL Codeine-D_3 / Morphine-D_3 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	20 µg/mL Codeine-D_3 / Morphine-D_3 internal standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

3240 ng/mL Morphine-3β-D-Glucuronide control working solution

Amount	Ingredients	Measuring Device
324 µL	1 mg/mL Morphine-3β-D-Glucuronide Cerilliant standard (M-031)	Pipette suitable for measuring 324 µL
QS to 100 mL	Negative urine	100 mL Class A volumetric flask

Hydrocodone Blood and Urine Stock and Working Solutions

100 µg/mL Hydrocodone calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Hydrocodone Cerilliant standard (H-003)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

5 µg/mL Hydrocodone calibration standard working solution

Amount	Ingredients	Measuring Device
500 µL	100 µg/mL Hydrocodone calibration standard stock solution	Pipette suitable for measuring 500 µL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

5 µg/mL Hydrocodone control working solution

Prepare a 5 µg/mL Hydrocodone control working solution using a similar formulation as described for preparing the 5 µg/mL Hydrocodone calibration standard working solution, with the exception that the control material must come from a different manufacturer than Cerilliant.

2 µg/mL Hydrocodone-D_3 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Hydrocodone-D_3 Cerilliant standard (H-005)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 50 mL	Methanol	50 mL Class A volumetric flask

Phencyclidine Blood and Urine Stock and Working Solutions

100 µg/mL PCP Cerilliant calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL PCP Cerilliant standard (P-007)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL PCP Cerilliant calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL PCP Cerilliant calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1 µg/mL PCP Cerilliant calibration standard working solution

Amount	Ingredients	Measuring Device
1 mL	10 µg/mL PCP Cerilliant calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

1 µg/mL PCP control working solution

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

10 µg/mL PCP-D_5 Cerilliant internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL PCP-D_5 Cerilliant standard (P-003)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

2 µg/mL PCP-D_5 Cerilliant internal standard working solution

Amount	Ingredients	Measuring Device
2 mL	10 µg/mL PCP-D_5 Cerilliant internal standard stock solution	1 mL Class A micro volumetric flask or pipette(s) suitable for measuring 2 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

Benzodiazepine Blood and Urine Stock and Working Solutions

100 µg/mL Nordiazepam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Nordiazepam Cerilliant standard (N-905)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Oxazepam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Oxazepam Cerilliant standard (O-902)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Diazepam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Diazepam Cerilliant standard (D-907)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Lorazepam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Lorazepam Cerilliant standard (L-901)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Acetonitrile	10 mL Class A volumetric flask

100 µg/mL Temazepam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Temazepam Cerilliant standard (T-907)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Clonazepam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Clonazepam Cerilliant standard (C-907)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Alprazolam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Alprazolam Cerilliant standard (A-903)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Triazolam calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Triazolam Cerilliant standard (T-910)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

2 µg/mL Nordiazepam / Oxazepam / Diazepam / Lorazepam / Temazepam / Clonazepam / Alprazolam / Triazolam calibration standard working solution

Amount	Ingredients	Measuring Device
200 µL	100 µg/mL Nordiazepam calibration standard stock solution	Pipette suitable for measuring 200 µL
200 µL	100 µg/mL Oxazepam calibration standard stock solution	Pipette suitable for measuring 200 µL
200 µL	100 µg/mL Diazepam calibration standard stock solution	Pipette suitable for measuring 200 µL
200 µL	100 µg/mL Lorazepam calibration standard stock solution	Pipette suitable for measuring 200 µL
200 µL	100 µg/mL Temazepam calibration standard stock solution	Pipette suitable for measuring 200 µL
200 µL	100 µg/mL Clonazepam calibration standard stock solution	Pipette suitable for measuring 200 µL

200 µL	100 µg/mL Alprazolam calibration standard stock solution	Pipette suitable for measuring 200 µL
200 µL	100 µg/mL Triazolam calibration standard stock solution	Pipette suitable for measuring 200 µL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

2 µg/mL Nordiazepam / Oxazepam / Diazepam / Lorazepam / Temazepam / Clonazepam / Alprazolam / Triazolam control working solution

Prepare a 2 µg/mL Nordiazepam / Oxazepam / Diazepam / Lorazepam / Temazepam / Clonazepam / Alprazolam / Triazolam control working solution using a similar formulation as the one described for preparing the 2 µg/mL Nordiazepam / Oxazepam / Diazepam / Lorazepam / Temazepam / Clonazepam / Alprazolam / Triazolam calibration standard working solution, with the exception that the control material must originate from a different manufacturer than the Certified Reference Material used to prepare the calibration standard working solution.

10 µg/mL Nordiazepam / Oxazepam / Lorazepam / Temazepam / α-Hydroxyalprazolam / α-Hydroxytriazolam calibration standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Nordiazepam calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Oxazepam calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Lorazepam calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Temazepam calibration standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL α-Hydroxyalprazolam Cerilliant standard (A-905)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL α-Hydroxytriazolam Cerilliant standard (T-915)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL Nordiazepam / Oxazepam / Lorazepam / Temazepam / α-Hydroxyalprazolam / α-Hydroxytriazolam control working solution

Prepare a 10 µg/mL Nordiazepam / Oxazepam / Lorazepam / Temazepam / α-Hydroxyalprazolam / α-Hydroxytriazolam control working solution using a similar formulation as the one described for preparing the 10 µg/mL Nordiazepam / Oxazepam / Lorazepam / Temazepam / α-Hydroxyalprazolam / α-Hydroxytriazolam calibration standard working solution, with the exception that the control material must originate from a different manufacturer than Cerilliant.

20 µg/mL Nordiazepam-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Nordiazepam-D_5 Cerilliant standard (N-903)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

20 µg/mL Oxazepam-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Oxazepam-D_5 Cerilliant standard (O-901	1 mL Class A micro volumetric flask a pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

20 µg/mL Diazepam-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Diazepam-D_5 Cerilliant standard (D-902	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

20 µg/mL Lorazepam-D_4 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Lorazepam-D_4 Cerilliant standard (L-902)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Acetonitrile	5 mL Class A volumetric flask

20 µg/mL Temazepam-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Temazepam-D_5 Cerilliant standard (T-902)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

20 µg/mL Clonazepam-D_4 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Clonazepam-D_4 Cerilliant standard (C-905)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

20 µg/mL Alprazolam-D_5 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Alprazolam-D_5 Cerilliant standard (A-902)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

20 µg/mL Triazolam-D_4 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Triazolam-D_4 Cerilliant standard (T-908)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 5 mL	Methanol	5 mL Class A volumetric flask

2 µg/mL Nordiazepam-D_5 / Oxazepam-D_5 / Diazepam-D_5 / Lorazepam-D_4 / Temazepam-D_5 / Clonazepam-D_4 / Alprazolam-D_5 / Triazolam-D_4 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	20 µg/mL Nordiazepam-D_5 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	20 µg/mL Oxazepam-D_5 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	20 µg/mL Diazepam-D_5 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL

1 mL	20 µg/mL Lorazepam-D_4 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	20 µg/mL Temazepam-D_5 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	20 µg/mL Clonazepam-D_4 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	20 µg/mL Alprazolam-D_5 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	20 µg/mL Triazolam-D_4 internal standard stock solution solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL Nordiazepam-D_5 / Oxazepam-D_5 / Lorazepam-D_4 / Temazepam-D_5 / α-Hydroxyalprazolam-D_5 / α-Hydroxytriazolam-D_4 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Nordiazepam-D_5 Cerilliant standard (N-903)	1 mL Class A micro volumetric flask pipette suitable for measuring 1 mL
1 mL	100 µg/mL Oxazepam-D_5 Cerilliant standard (O-901)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Lorazepam-D_4 Cerilliant standard (L-902	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL Temazepam-D_5 Cerilliant standard (T-902)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL α-Hydroxyalprazolam-D_5 Cerilliant standard (A-904)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	100 µg/mL α-Hydroxytriazolam-D_4 Cerilliant standard (T-909)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

10 µg/mL Oxazepam Glucuronide control working solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL Oxazepam Glucuronide Cerilliant standard (O-023)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Distilled water	10 mL Class A volumetric flask

Carisoprodol / Meprobamate Blood Stock and Working Solutions

100 µg/mL Carisoprodol / Meprobamate calibration standard working solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL Carisoprodol Cerilliant standard (C-077)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
1 mL	1.0 mg/mL Meprobamate Cerilliant standard (M-039)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

100 µg/mL Carisoprodol / Meprobamate control working solution

Prepare a 100 µg/mL Carisoprodol / Meprobamate control working solution using a similar formulation described for preparing the 100 µg/mL Carisoprodol / Meprobamate calibration standard working solution, with the exception that the control material must originate from a different manufacturer than Cerilliant.

20 µg/mL Carisoprodol-D_7 / Meprobamate-D_7 internal standard working solution

Amount	Ingredients	Measuring Device
2 mL	100 µg/mL Carisoprodol-D_7 Cerilliant standard (C-083)	1 mL Class A micro volumetric flask or pipette(s) suitable for measuring 2 mL
2 mL	100 µg/mL Meprobamate-D_7 Cerilliant standard (M-131)	1 mL Class A micro volumetric flask or pipette(2) suitable for measuring 2 mL
QS to 10 mL	Methanol	10 mL Class A volumetric flask

6-Acetylmorphine Urine Stock and Working Solutions

100 µg/mL 6-Acetylmorphine calibration standard stock solution

Amount	Ingredients	Measuring Device
1 mL	1.0 mg/mL 6-Acetylmorphine Cerilliant standard (A-009)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Acetonitrile	10 mL Class A volumetric flask

2 µg/mL 6-Acetylmorphine calibration standard working solution

Amount	Ingredients	Measuring Device
200 µL	100 µg/mL 6-Acetylmorphine calibration standard stock solution	Pipette suitable for measuring 200 µL
QS to 10 mL	Acetonitrile	10 mL Class A volumetric flask

2 µg/mL 6-Acetylmorphine control working solution

Prepare a 2 µg/mL 6-Acetylmorphine control working solution using a similar formulation as described for preparing the 2 µg/mL 6-Acetylmorphine calibration standard working solution, with the exception that the control material must come from a different manufacturer than Cerilliant.

10 µg/mL 6-Acetylmorphine-D_3 internal standard stock solution

Amount	Ingredients	Measuring Device
1 mL	100 µg/mL 6-Acetylmorphine-D_3 Cerilliant standard (A-006)	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Acetonitrile	10 mL Class A volumetric flask

1 µg/mL 6-Acetylmorphine-D_3 internal standard working solution

Amount	Ingredients	Measuring Device
1 mL	10 µg/mL 6-Acetylmorphine-D_3 internal standard stock solution	1 mL Class A micro volumetric flask or pipette suitable for measuring 1 mL
QS to 10 mL	Acetonitrile	10 mL Class A volumetric flask

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4.4 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, with the exception of 1% acidic methanol and β-glucuronidase enzyme solution in 100 mM acetate buffer pH 5.0 (5,000 units/mL), 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.

When weighing chemicals for reagents, weights will be truncated to the appropriate amount of places listed in the recipes.

ACIDIC SOLUTIONS

Acetic acid, 1.0 M

- Add 5.76 mL of glacial acetic acid to distilled 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 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.

Hydrochloric acid, 100 mM

- Add 2.1 mL of concentrated hydrochloric acid to distilled water and dilute to 250 mL.
- QC: Check pH with pH paper. pH should be less than 7.
- Storage: Room temperature in glass or plastic container.

- Stability: 6 months.

BASIC SOLUTIONS

Sodium hydroxide 10 N

- Slowly dissolve 24 g of NaOH in 50 mL of distilled water.
  CAUTION – EXOTHERMIC REACTION!
- Allow to cool and dilute to 60 mL.
- QC: Check pH with pH paper. pH should be greater than 7.
- Storage: Room temperature in plastic container.
- Stability: 6 months.

Sodium hydroxide 1 N

- Dilute 10 mL of 10 N sodium hydroxide to 100 mL with distilled water.
- QC: Check pH with pH paper. pH should be greater than 7.
- Storage: Room temperature in plastic container.
- Stability: 6 months

BUFFER SOLUTIONS

Acetate buffer, 100 mM, pH 4.5

- Dissolve 5.86 g of sodium acetate trihydrate in 975 mL of distilled water. Add 3.24 mL of glacial acetic acid. Dilute to 1 liter with distilled water. Adjust pH to 4.5 ± 0.1 with 1 M acetic acid or 1 N NaOH.
- QC: Check pH with pH paper.
- Storage: Refrigerate in glass container.
- Stability: 6 months. Inspect for contamination before use.

Acetate buffer, 100 mM, pH 5.0

- Dissolve 2.145 g sodium acetate trihydrate in ~230 mL distilled water. Add 0.52 mL glacial acetic acid. Dilute to 250 mL. Adjust pH to 5.0 ± 0.1 with 1M acetic acid or 1 N NaOH.
- QC: Check pH with pH paper.
- Storage: Room temperature in glass or plastic container.
- Stability: 6 months. Inspect for contamination before use.

Carbonate buffer

- Dissolve 2 g potassium bicarbonate ($KHCO_3$) and 1 g potassium carbonate – 1.5 Hydrate (K_2CO_3 - $1.5H_2O$) in 75 mL of distilled water. Dilute to 100 mL.
- Prepare fresh daily for one time use.
- QC: Concurrently with use. All drug confirmation Batch Acceptance Criteria must be met for passing QC.

Phosphate buffer, 100 mM, pH 6.0

- Dissolve 1.70 g sodium phosphate dibasic and 12.14 g sodium phosphate monobasic in distilled 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.

ENZYME SOLUTION

β-glucuronidase enzyme solution in 100 mM acetate buffer pH 5.0 (5,000 units/mL)

- Prepare fresh daily for one time use.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- If the enzyme activity is listed as greater than or equal, the lowest enzyme activity will be used for the calculation in order to achieve the final enzyme activity of 5000 units/mL.
- Use the following formula to determine the volume of β-glucuronidase enzyme needed:

$$\text{Volume of glucuronidase} = \frac{5000\frac{\text{units}}{\text{mL}} \text{ x Volume of solution}}{\text{Enzyme activity of glucuronidase}}$$

- Example: If 20 mL of β-glucuronidase enzyme solution in 100 mM acetate buffer pH 5.0 is needed to be made from β-glucuronidase enzyme with enzyme activity of greater than or equal to 100000 units/mL, the volume of glucuronidase enzyme is determined as follows.

$$\text{Volume of glucuronidase} = \frac{5000\frac{\text{units}}{\text{mL}} \text{ x } 20 \text{ mL}}{100000\frac{\text{units}}{\text{mL}}} = 1 \text{ mL or } 1000 \text{ µL}$$

LC/MS/MS REAGENTS

50/50 Water/Methanol (Needle Rinse)

- Prepare a 50/50 mixture of LCMS Grade Water/LCMS Grade Methanol in a LCMS glass reagent bottle (e.g., using a LCMS glass reagent bottle for measuring, combine 200 mL of LCMS Grade Water and 200 mL of LCMS Grade Methanol into the LCMS reagent bottle). Swirl to mix.
- QC: Not applicable. Reagent is used as a wash solution only.
- Storage: Room temperature in LCMS glass reagent bottle.

1:1:1:1 Methanol:Water:Acetonitrile:2-Propanol (Needle Rinse)

- 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.

5 M Ammonium Acetate Stock Solution

- Using a 20 mL Class A volumetric flask, dissolve 7.708 g of LCMS Grade Ammonium Acetate in 20 mL of LCMS Grade Water. Mix.
- Calculation:

$$\frac{5 \text{ mole Ammonium Acetate}}{\text{L}} \times \frac{77.08 \text{ g Ammonium Acetate}}{\text{mole Ammonium Acetate}} \times 0.020 \text{ L} = 7.708 \text{ g Ammonium Acetate}$$

- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.
- Storage: Refrigerate in amber glass bottle(s).

5 mM Ammonium Acetate in Water (Mobile Phase)

- Prepare a 5 mM Ammonium Acetate in Water Solution into a LCMS amber glass reagent bottle (e.g., using a LCMS amber glass reagent bottle for measuring, add 500 mL of LCMS Grade Water to the LCMS amber glass reagent bottle; pipette 500 µL of a 5 M Ammonium Acetate Stock Solution to the water). Swirl to mix.
- QC: Concurrently with batch. All drug confirmation Batch Acceptance Criteria must be met for passing QC.

- Storage: Room temperature in LCMS amber glass reagent bottle.

0.1% Formic Acid in Water (Mobile Phase)

- 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.

LVMPD FORENSIC LABORATORY TECHNICAL PROCEDURES TOXICOLOGY

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 examiner 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 #3. 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, analyst's initials, and vial position numbers as it pertains to the Blood Alcohol

Sequence Table.

4) Invert the blood tube several times and/or vortex to re-suspend the blood cells.
5) Compare the Lab number/item number and vial position numbers on the blood tube label with the Lab number/item number and vial position numbers 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 > 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 his/her 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 ≤

0.4000 g/100mL. For 0.0200 g/100mL ≤ BAC < 0.0500 g/100mL, the highest result must be no greater than ± 0.0050 g/100mL of the lowest result. For BAC < 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 < 0.020 g/100mL, report “a concentration of alcohol of less than 0.020 g/100ml of blood”. If the BAC < 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 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 < 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 < 0.020 g/100mL) but does not identify ethanol in one or more of the other results (i.e., BAC = 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 ≤ 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.

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: 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

C. Reagents: 1-propanol, ACS grade or better.
Distilled 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 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 water to the container.

5. Mix the internal standard working solution.

6. Label the container in accordance with section 5.1.3.1 of the LVMPD Forensic Handbook.

7. Transfer to reagent bottles. Label the reagent bottles in accordance with section 5.1.3.1 of the LVMPD Forensic Handbook.

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 *5.5 Ethanol Analysis Quality Assurance*.

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5.2 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 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 water.

3. Transfer to an appropriate container. Label in accordance with section 5.1.3.1 of the Forensic Handbook.

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 *5.5 Ethanol Analysis Quality Assurance*.

LVMPD FORENSIC LABORATORY
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5.3 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)

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) 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 of ethanol. Note that the amount of measured ethanol does not need to be exact.

Target ethanol concentration	Target weight of ethanol
0.060 g/100mL	0.0120 g
0.080 g/100mL	0.0160 g
0.100 g/100mL	0.0200 g

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

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 following formula:

   (grams of ethanol)/(20 mL) x (100 mL) = (x grams ethanol/100 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)

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 *5.5 Ethanol Analysis Quality Assurance*.

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5.4 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)

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 of ethanol. Note that the amount of measured ethanol does not need to be exact.

Target ethanol concentration	Target weight of ethanol
0.060 g/100mL	0.0120 g
0.080 g/100mL	0.0160 g
0.100 g/100mL	0.0200 g

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

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:

   (grams of ethanol)/(20 mL) x (100 mL) = (x grams ethanol/100 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)

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 *5.5 Ethanol Analysis Quality Assurance*.

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5.5 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 forth 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 QC packet. Indicate the QC method (GCHS), “passed” or “failed”, and your initials. Store the QC packet 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 forth decimal place, must be no greater than 0.02 g/100mL.

3) Record your results on the QC packet. Indicate the QC method (GCHS), "passed" or "failed", and your initials. Store the QC packet 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 in the LIMS Resource Manager 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 in the LIMS Resource Manager 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 against a valid calibration prior to being used for casework analysis.

2) Interpretation: All results for ethanol must be 0.000 g/dL.

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

2) Interpretation: All results for ethanol must be 0.000 g/dL.

3) Record your results on the QC packet. Indicate the QC method (GCHS), “passed” or “failed”, and your initials. Store the QC packet 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 in the LIMS Resource Manager 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 forth 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 QC packet. Indicate the QC method (GCHS), “passed” or “failed”, and your initials. Store the QC packet 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.

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) is the date the reagent was prepared) (See Forensic Handbook sec. 5.1.3.1).
- 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

6.3 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.

6.4 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.

6.4.1 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.

6.4.2 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 (e.g., ELISA or EMIT) 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. All QC criteria stated in the Batch Acceptance Criteria must be met. In addition, optical density/absorbance values should be comparable to previous calibrator or control results.

Confirmation working solutions (e.g., GC/MS or LC/MS/MS) 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. Note that quality control checks of new lots of Codeine/Morphine urine, Benzodiazepine urine and THCA urine standard working solutions must include the corresponding glucuronide control. The entire extraction methodology must be used.

Newly prepared lots of internal standard working solutions are quality control checked using the applicable Confirmation method (e.g., GC/MS or LC/MS/MS) 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.

6.5 Controls

Positive controls are included in casework batches to monitor the calibration of each batch. Positive control data from casework batches are logged into spreadsheets and reviewed for trends by the analyst entering the data. The analysts will indicate that they have checked the data for trends by entering their initials onto the spreadsheet. Statistical techniques are applied to the blood alcohol and drug confirmation data when measurement uncertainty is updated.

Spreadsheets are located at:
H:\CB\Forensics\Toxicology\SCREENS\Blood Screens--DSX\Blood Screen QC Stats
H:\CB\Forensics\Toxicology\SCREENS\Urine Screens--VIVA-E\Urine Screen QC Stats
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.

6.6 Negative Matrix

6.6.1 Negative Blood

Negative whole blood may be purchased from an outside vendor or supplied in-house.

6.6.1.1 Quality Control

ELISA - Analyze in duplicate against the current lot of working stock solution on a screening batch. 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 – Because amphetamine, clonazepam, and lorazepam have low cross-reactivity with the ELISA screen, and these drugs are confirmed if they are listed as suspected drugs on the Toxicology Request Form, new lots of negative whole blood should also be verified on the AMP/METH/MDA/MDMA in Blood and Benzodiazepines in Blood confirmation methods prior to analyzing casework samples. The negative whole blood sample should be extracted in duplicate with internal standard on a valid curve. Negative whole blood samples should show no indications of the target drugs.

Blood Ethanol – See Chapter 5.5 Ethanol Analysis Quality Assurance for QC requirements.

6.6.2 Negative Urine

Negative Urine may be purchased from an outside vendor or supplied in-house.

6.6.2.1 Quality Control

EMIT - Analyze on the Viva-E in duplicate for at least a 7-panel plus adulterants. Negative urine must produce negative results for all drugs and normal results for adulterants.

Drug Confirmation – Because lorazepam has low cross-reactivity on the EMIT screen, and this drug is confirmed if it is listed as a suspected drug on the Toxicology Request Form, new lots of negative urine should also be verified on the Benzodiazepines in Urine confirmation method prior to analyzing casework samples. The negative urine sample should be extracted in duplicate with internal standard on a valid curve. Negative urine samples should show no indications of the target drug.

Urine Ethanol - See Chapter 5.5 Ethanol Analysis Quality Assurance for QC requirements.

6.7 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 Guide 34:20094 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.

6.8 Chemical and Drug Inventory

Certified reference materials of quantitative solutions of drugs and metabolites are available from various vendors. Since these samples are consumed upon opening,

they will not be documented in the Chemical Inventory. 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.

6.9 Expiration Dates

The expiration date of a chemical/reagent/reference material is defined as the manufacturer's expiration/use by/retest/minimum shelf life date, with exceptions noted below, or elsewhere in this manual. Chemicals/reagents/reference materials will not be used beyond the expiration date. If a chemical/reagent/reference material 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.

6.9.1 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.

6.9.2 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.

6.9.3 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.

6.9.4 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 4.3.

6.9.5 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.

6.10 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.

6.10.1 GC/MS

GC/MS 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. GC/MS employed in chemical ionization modes should be tuned after the installation of the chemical ionization source. Additional tuning in chemical ionization modes is performed at the operator's discretion. Tuning data will be maintained for one year at the instrument site.

6.10.2 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.

6.10.3 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.

6.10.4 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.

6.11 Measuring Equipment

6.11.1 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.

6.11.2 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.

6.11.2.1 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.

6.12 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

6.12.1 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.

6.12.2 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

Prior to preparing standards, controls, internal standards, and EMIT calibrators and controls, the preparer will rinse the glassware with methanol followed by the solvent which will be used in the preparation. Care will be taken to ensure that micro volumetric flasks are dry prior to measuring reference materials.

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

6.13 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, calibrators and controls to come to room temperature prior to use.
- Bubbles present in samples.
- Use of pipettes which are not in working order.
- Boric acid is not recommended as a preservative for urine.
- Sediment in urine samples.

- 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.

6.14 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
- Complete one supervised/technically reviewed 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.

6.15 Back-up of Electronic Records

Instrumental data will be backed up on an annual basis onto an assigned external hard drive. The external hard drive is stored in the Toxicology Lab.

6.16 Technical Reviews

Technical Reviews are required to be conducted on all cases.

6.17 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)*

6.18 Proficiency Tests

6.18.1 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.

6.18.2 Passing Criteria for Proficiency Tests

6.18.2.1 External Tests

Vendor - College of American Pathologist (CAP)
Tests - Alcohol (blood) and drugs (blood and urine)
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

6.18.2.2 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

LVMPD FORENSIC LABORATORY
TECHNICAL PROCEDURES
TOXICOLOGY

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.

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

   SYRINGE: 250 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). 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 #8 ThermoFisher Scientific Refrigerator Model: MH49SS-GAEE-VW SN: 0146287301120307			
	Tox #9 Isotemp Plus Refrigerator Fisher Scientific Model: MR72SS-GAEE-FS SN: 0142034601150928			
	Tox #10 Whirlpool Refrigerator/Freezer Model: WRT106TFDW01 SN: VS64638614			

	Instrument	Frequency	Criteria	Corrective Action
	Tox #11 True Refrigerator Model: GDM-49-SCI-HC_TSL01 SN: 9255406			
Pipettes	Pipette identifications and certification schedules are listed in Toxicology pipette logbook maintained by Toxicology Detail Calibration plan is located at H:\CB\Forensics\Toxicology\Pipettes\Diluter-Pipette Calibration Reference	**External:** Calibrate annually **Critical Service** 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. 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% should not be greater than 3.000 on the Artel® Pipette Tracker™. Volume ≥ 15 µL +/- 3% (actual inaccuracy). CV% 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.
Balances	Tox #3 Mettler Model: XS105DU SN: 1127113910	**External:** Calibrate annually **Critical Service** Vendor options: Mettler Toledo, Inc. (800) 523-5123	**External:** See the Toxicology Balances Calibration Information located at H:\CB\Forensics\Toxicology\Balances for the minimum required levels of	If a balance is not operating properly: 1. Initiate manufacturer's procedures to perform a mechanical internal calibration (if applicable)

	Instrument	Frequency	Criteria	Corrective Action
	Tox #5 Ohaus Model: SPX222 SN: B618431040	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.	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.	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 Tox #6 LABCONCO Model: 7280400 SN: 050639181H	**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
Critical Thermometers (*See Refrigerator/Freezer Temperature Log for location information)	VWR Model No: 61161-364 SN: 180056711*	**External:** N/A	N/A	N/A
	VWR Model No: 61161-364 SN: 180056713*	NIST Thermometers – Replace every two years or sooner per manufacturer's guidelines.		
	VWR Model No: 61161-364 SN: 180056714*			
	VWR Model No: 61161-364 SN: 180056715*			
	VWR Model No: 61161-364 SN: 180056716*			
	VWR Model No: 61161-364 SN: 180056717*			
	VWR Model No: 61161-364 SN: 180056720*			
	VWR Model No: 61161-364 SN: 180056721*			
	VWR Model No: 61161-364 SN: 180056722*			
	VWR Model No: 61161-364 SN: 180056724*			
	VWR Model No: 61161-364 SN: 180056727*			

	Instrument	Frequency	Criteria	Corrective Action
	VWR Model No: 61161-364 SN: 180056729*			
	VWR Model No: 61161-364 SN: 180056732*			
Non-Critical Thermometers	(Tox #1 Oven) VWR Model No: 76204-528 SN: 181232870	**External:** N/A NIST Thermometers – Replace every two years or sooner per manufacturer's guidelines	N/A	N/A
	(Tox #2 Heat Block) VWR Model No: 61161-310 SN: 181098640			
	(Tox #3 Heat Block) VWR Model No: 61161-310 SN: 181098650			
Diluter/Dispensers	Tox #6 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600BD1621 Controller SN: MD600BC1527	**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	**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	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.
	Tox #7 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600GH10521 Controller SN: ML600GG10491			

	Instrument	Frequency	Criteria	Corrective Action
	Tox #8 Diluter Hamilton Model: MicroLab 600 Driver SN: ML600GJ10733 Controller SN: ML600GH10667	**Internal:** When in use: Conduct a performance check of the syringe monthly, replace as needed. Verification must be done every time any parts are replaced. After send-out repair: Conduct a performance 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.	volume checked Use "Hamilton Diluter/Dispenser Performance Check Record" Form located in Qualtrax. Logbooks and Verifications are located in Resource Manager.	
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.
GC/MS	Tox #9 GCMS GC Model: Agilent 7890A SN: CN10521043 MS Model: Agilent 5975C SN: US10523720	**External:** Refer to specific instrument contract/agreement for appropriate support phone numbers and service agreement	**External**: Meet external vendor criteria. **Internal:** **MS performance -** Autotune must be performed each day that	At a minimum, attempt the following corrective action if any of the performance checks fail: 1. Repeat test. 2. Troubleshoot using manufacturer's

	Instrument	Frequency	Criteria	Corrective Action
	Tox #10 GCMS GC Model: Agilent 7890A SN: CN10501124 MS Model: Agilent 5975C SN: US10494606 (CI mode capability)	**Internal:** Before use (once/day) and after send-out repair.	the instrument will be used, prior to analysis. N_2 should not be greater than 10% for EI mode. **Note:** Criteria does not apply to Tox # 10 instrument when in CI 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.	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 LCMS 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 –** Autotune or checktune must be performed each day the instrument will be used, prior to analysis. The tune must be performed in the mode used for 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). Logbook is located in lab area near equipment.	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 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	**External:** Meet external vendor criteria. **Internal:** Daily maintenance as outlined in the technical manual.	At a minimum, attempt the following corrective action if any of the performance checks fail: 1. Repeat test. 2. Troubleshoot using manufacturer’s

	Instrument	Frequency	Criteria	Corrective Action
	Siemens EMIT Analyzer Model: Viva-E SN: 15-2411	Orasure Technologies Inc (800) 869-3538 Dade-Behring (800) 227-8994 **External (EMIT Only):** Preventative maintenance semi-annually **Internal (EMIT Only):** before use (once/day), weekly & monthly & quarterly	Dynex Only: A self test must be performed each day the instrument will be used, prior to analysis. EMIT Only: Maintain **weekly & monthly** cleaning schedule. Use Weekly/Monthly and daily Maintenance Forms found in Qualtrax. Logbooks are located in lab area near equipment.	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.
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. Instrument/Maintenance Logbooks are located in lab area near equipment (Internal criteria checks are kept with batch data separate from the instrument logbook).	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
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 Generators	HG #5 Parker Hannifin Model: H2PEM-510-L1466 SN: 12PHG5185 HG #6 Parker Hannifin Model: H2PEM-510-L1466 SN: 15PHG5058 HG #7 Parker Hannifin Model: H2PEM-510-L1466 SN: 17PHG5O66	**External:** None **Internal:** Every 6 Months Replace parts contained in vendor 6 month maintenance kit	**Internal:** Maintains pressure and produces hydrogen gas. Logbooks are located in lab area near equipment. Hydrogen Generator Water Check forms (document number 13269) are stored 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.
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. Logbook is located in lab area near equipment.	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.

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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)

Viva-E

Siemens Viva-E Operators Manual

WinTox6 Basic User Guide (iMS) 6.8.32

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

Siemens Package Inserts

ASB Standard 017, First Edition 2017, Standard Practices for Measurement Traceability in Forensic Toxicology – Draft

ASB Standard 036, First Edition 2017, Standard Practices for Method Validation in Forensic Toxicology - Draft

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9.1 Title: **APPENDIX - ABBREVIATIONS KEY**

Abbreviations Key

⊕, POS - positive
⊖, NEG - negative
ACQ – acquisition
Adult - adulterants
AG, AGY - agency
ALP- alprazolam
AM, AMP, AMPH – amphetamine
BA – blood alcohol
B/C - barcode
BENZO, BENZ, BZ, Benzodiazep – benzodiazepines
BLNK, BLK – blank
BSTFA - N,O-Bis(trimethylsilyl)trifluoroacetamide
BZE - benzoylecgonine (cocaine metabolite)
CAL – calibrator
CALIB - calibration
CARI, CAR – carisoprodol
CHROM - Chromium
CF – correction factor
CLON - clonazepam
COC, COCN, C – cocaine
CO, CU, CUT, C/O - cutoff
COD – codeine
CR, CREAT – creatinine
CRM – certified reference material
CTRL, CTL – control
CV – coefficient of variation
EV # or EN – event number
dAbs/m – delta absorbance per meter
DA – District Attorney
DEF – deferred
DI - deionized
DIAZ – diazepam
DS – drug screen
EBT – evidential breath test
EI – electron impact
ELISA – enzyme-linked immunosorbent assay
EMIT – enzyme-multiplied immunoassay technique
ESI – electrospray ionization

ETHAN, EtOH - ethanol
EVI – evidence
EXP – expiration
FA – further analysis
FA – Forensic Advantage
FN – first name
FRED - Forensic Request and Examination Database
GC – gas chromatograph
GCHS – headspace gas chromatograph
GCMS, GC/MS – gas chromatograph/mass spectrometer
GHB – gamma hydroxy-butyrate
HI – high
HMDS - Bis(trimethylsilyl)amine aka hexamethyldisilazane
HYC – hydrocodone
IMM - Immunalysis
INT STD, ISTD, IS – internal standard
KIO - kit individually opened
LC – liquid chromatography
LCMS, LC/MS – liquid chromatography/mass spectrometry
LC/MS/MS, LC/MS QQQ – liquid chromatography tandem mass spectrometry
LIMS - Laboratory Information Management System
LN – last name
LORAZ - lorazepam
MDA - 3,4-methylenedioxyamphetamine
MDMA - 3,4-methylenedioxymethamphetamine
MEPRO, MEP - meprobamate
METH, MAMP – methamphetamine
6-AM, 6-MAM – 6- acetyl morphine
MOR – morphine
MRM – multiple reaction monitoring
MSD – mass selective detector
N-PROP – n-propanol
N/A – not applicable
NCAL – negative calibrator
NCS – no controlled substances
NFA – no further analysis
NM - name
NORDIAZ – nordiazepam
NT, NIT - nitrate
OH-ALP - α-hydroxyalprazolam
OH-TRIAZ - α-hydroxytriazolam
OD – optical density
OF – oral fluid
OR - object repository
ORS - OraSure
OPI, OP, OPIA – opiates
OX - oxidant
OXAZ – oxazepam

OXC, OXY, OXYC - oxycodone
OXM - oxymorphone
PBT – preliminary breath test
PC - Property Connect
PCP - phencyclidine
PC Sgt. - Property Crimes Sergeant
PFAA - pentafluoropropionic acid anhydride
PFTBA – perfluorotributylamine
PTFE - polytetrafluoroethylene
PI – personal identifiers
PN – part number
POI - persons of interest
PREP – preparation
PSI – pounds per square inch
QC – quality control
QNS – quantity not sufficient
QQQ – triple quadrupole
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
TARG - target
TEMAZ – temazepam
TFE - tetrafluoroethylene
T, THC - Δ^9-tetrahydrocannabinol
THCA - 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (Marijuana metabolite)
TIC – total ion chromatogram
TOX - toxicology
DS DEF – deferred from drug screening
TMCS - trimethylchlorosilane
TRIAZ – triazolam
UC – until consumed
UR - unit record, urine
V- volts, volume

WB – whole blood
WBC – whole blood control
XTC, EX – ecstasy (see MDMA)

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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
GC/HS	**GC#3, GC #4, GC #5**	TotalChrom Workstation 6.3.2.0646
Immunoassay	**Dynex DSX ELISA**	Revelation: v.6.24
Immunoassay	**Siemens EMIT®**	Syva Viva-E Analyzer: v.2.0.14
Immunoassay	**Siemens EMIT®**	WinTox 6: v.6.8.79-0
Pipette Calibration System	**ARTEL Pipette Tracker®**	v3.3