



# ARKANSAS STATE CRIME LABORATORY



## *FORENSIC TOXICOLOGY QUALITY MANUAL*

**Executive Director:**  
*Kermit B. Channell II*

# TABLE OF CONTENTS

<b>SECTION 1: INTRODUCTION.....</b>	<b>1-1</b>
1.1: GOALS AND OBJECTIVES .....	1-1
1.2: QUALITY MANUAL.....	1-1
<b>SECTION 2: PERSONNEL QUALIFICATIONS AND TRAINING.....</b>	<b>2-1</b>
2.1: MINIMUM QUALIFICATIONS.....	2-1
2.2: JOB DESCRIPTION .....	2-1
2.3: TRAINING.....	2-1
<b>SECTION 3: FACILITIES AND SECURITY .....</b>	<b>3-1</b>
3.1: SECTION FACILITIES.....	3-1
3.2: SECTION SECURITY.....	3-1
<b>SECTION 4: EVIDENCE CONTROL AND CASE MANAGEMENT .....</b>	<b>4-1</b>
4.1: INTRODUCTION .....	4-1
4.2: ACCESSION OF EVIDENCE .....	4-1
4.3: STORAGE.....	4-1
4.4: CASE PRIORITY POLICY .....	4-2
4.5: DISPOSAL .....	4-2
4.6: RELEASE.....	4-2
4.7: CESSATION OF ANALYSIS .....	4-2
4.8: CASES INAPPROPRIATE FOR ANALYSIS.....	4-2
4.9: EVIDENCE MARKING AND SEALING .....	4-3
4.10: INTER-LABORATORY EVIDENCE TRANSFER.....	4-3
<b>SECTION 5: VALIDATION .....</b>	<b>5-1</b>
<b>SECTION 6: ANALYTICAL PROCEDURES.....</b>	<b>6-1</b>
6.1: INTRODUCTION .....	6-1
6.2: GENERAL REQUIREMENTS.....	6-1
6.3: COURSE OF ANALYSIS .....	6-1
6.3.1: <i>Initial Screening</i> .....	6-1
6.3.2: <i>Law enforcement cases</i> .....	6-2
6.3.2.1: DUI cases.....	6-2
6.3.2.2: Other cases.....	6-2
6.3.2.3: ME cases .....	6-3
6.4: ACCEPTABLE WORK PRODUCT .....	6-3
6.4.1: <i>General Requirements</i> .....	6-3
6.4.2: <i>Controls</i> .....	6-4
6.4.3: <i>Quantitations</i> .....	6-4
6.4.4: <i>Notes</i> .....	6-5
6.4.5: <i>Additional aliquots</i> .....	6-5
6.4.6: <i>Additional Assays</i> .....	6-5
6.4.7: <i>Contamination countermeasures</i> .....	6-5
6.4.8: <i>Method specific requirements</i> .....	6-6
6.4.8.1: Gas Chromatography.....	6-6
6.4.8.2: Gas-Chromatography Mass-Spectrometry (GC-MS) .....	6-6
6.4.8.3: Carboximetry .....	6-7
6.4.8.4: AxSYM.....	6-7
6.4.8.5: ELISA .....	6-7
6.4.8.6: Reinsch .....	6-7
6.5: REPORTING .....	6-7
6.5.1: <i>General requirements</i> .....	6-7
6.5.2: <i>Language</i> .....	6-8
6.5.2.1: Disclaimers.....	6-8
6.6: STANDARDS AND REAGENTS.....	6-9
6.6.1: <i>General requirements</i> .....	6-9

6.6.2:	<i>Standards/controls</i> .....	6-9
6.6.3:	<i>Reagents</i> .....	6-10
6.6.3.1:	Purchased .....	6-10
6.6.3.2:	Prepared.....	6-10
6.7:	STANDARD OPERATING PROCEDURES – ANALYTICAL METHODS .....	6-11
6.7.1:	<i>AxSYM Screen</i> .....	6-11
6.7.2:	<i>Ethanol Analysis</i> .....	6-13
6.7.3:	<i>Base Screen</i> .....	6-16
6.7.4:	<i>Acid Screen</i> .....	6-19
6.7.5:	<i>Quantitation</i> .....	6-22
6.7.6:	<i>Urine Cannabinoid Confirmation</i> .....	6-23
6.7.7:	<i>Morphine and 6-Monoacetylmorphine with MBTFA (Liquid Extraction)</i> .....	6-25
6.7.8:	<i>Hydromorphone with MBTFA (Liquid Extraction)</i> .....	6-28
6.7.9:	<i>Reinsch Screen</i> .....	6-31
6.7.10:	<i>Cyanide screen and quantitation</i> .....	6-33
6.7.11:	<i>Carbon Monoxide</i> .....	6-35
6.7.11.1:	Carbon Monoxide by CO-Oximeter .....	6-35
6.7.11.2:	Carbon Monoxide by Diffusion Cell.....	6-37
6.7.11.3:	Carbon Monoxide by UV-Vis Spectrometer.....	6-39
6.7.12:	<i>Gamma-hydroxybutyrate (GHB) screen and quantitation</i> .....	6-41
6.7.13:	<i>Cocaine and Benzoyllecgonine by Solid Phase Extraction and Gas Chromatography-Mass Spectrometry</i> .....	6-44
6.7.14:	<i>ELISA Drug Screening</i> .....	6-47
6.7.15:	<i>Differentiation of Phentermine, Methamphetamine, Amphetamine, MDMA, and Pseudoephedrine by LC-MS-MS</i> .....	6-49
	STANDARD OPERATING PROCEDURES - OTHER METHODS .....	6-52
6.7.16:	<i>Analytical Calibration Curve Preparation</i> .....	6-52
6.7.17:	<i>Calibration Curve Evaluation</i> .....	6-52
<b>SECTION 7:</b>	<b>MAINTENANCE</b> .....	<b>7-1</b>
<b>SECTION 8:</b>	<b>PROFICIENCY TESTING PROGRAM</b> .....	<b>8-1</b>
8.1:	SOURCES OF TESTING .....	8-1
8.2:	EVALUATION OF RESULTS .....	8-1
<b>SECTION 9:</b>	<b>CASE RECORDS</b> .....	<b>9-1</b>
9.1:	CASE FILE STORAGE .....	9-1
9.2:	DATA RETENTION .....	9-1
<b>SECTION 10:</b>	<b>TESTIMONY REVIEW</b> .....	<b>10-1</b>
<b>SECTION 11:</b>	<b>AUDITS</b> .....	<b>11-1</b>
<b>SECTION 12:</b>	<b>HEALTH AND SAFETY</b> .....	<b>12-1</b>
<b>SECTION 13:</b>	<b>COMPLAINTS</b> .....	<b>13-1</b>
<b>SECTION 14:</b>	<b>MISCELLANEOUS</b> .....	<b>14-1</b>
14.1:	LITERATURE REVIEW .....	14-1
14.2:	GLOSSARY .....	14-1
14.3:	LIST OF STANDARD ABBREVIATIONS.....	14-2

## Section 1: Introduction

### 1.1: Goals and Objectives

The Forensic Toxicology Section of the Arkansas State Crime Laboratory was established by Arkansas Act 517 of 1977 as described in Arkansas Statute 12-12-301 and others (vide infra). The purpose of the Forensic Toxicology Section is to analyze biological specimens submitted to the Arkansas State Crime Laboratory for the presence of substances (e.g. alcohols, drugs, poisons) which may have contributed to a matter under investigation. A report of laboratory analysis is issued to the investigating officer or other person(s) as determined by laboratory policy.

#### ***12-12-301. Establishment.***

*(a) There is established a State Crime Laboratory.*

*(b) The laboratory shall offer services to law enforcement in pathology and biology, toxicology, criminalistics, raw drug analysis, latent fingerprint identification, questioned documents examination, firearms and toolmarks identification, and in other such areas as the State Crime Laboratory Board may deem necessary and appropriate.*

*History. Acts 1977, No. 517, §1; 1979, No. 864, §1; A.S.A. 1947, §42-1201.*

#### ***12-12-305. Housing and equipment - Functions.***

*(a) There shall be established under the supervision of the Executive Director of the State Crime Laboratory a central office and laboratory facility sufficient and adequate to house the various functions of the laboratory as set out in this subchapter and as may be necessary and proper for the laboratory to perform in carrying out its official duties and functions as provided by law.*

*(b) The laboratory shall have such equipment and personnel as is necessary to respond to the needs of all law enforcement agencies in the State of Arkansas with respect to the following functions:*

*(1) Forensic toxicology, which shall include, but is not limited to, chemical testing and analysis of body fluids and the performance of procedures to determine the presence and significance of toxic agents both in the investigation of death cases authorized by this chapter and in other appropriate cases;*

*(2) Criminalistics, which shall include, but is not limited to, chemical testing of trace evidence, physical and microscopic analysis of evidence, questioned document examination and classification, latent fingerprint identification and classification, firearms and toolmarks identification and analysis, and serology;*

*(3) Drug analysis, which shall include, but is not limited to, analyzing and identifying substances suspected as being controlled, illicit, or contraband drugs;*

*(4) Pathology and biology, which shall investigate and make a determination of the cause and manner of deaths which become subject to the jurisdiction of the State Medical Examiner as set out in §12-12-315 and shall include the general application of the medical sciences to assist the criminal justice system in the State of Arkansas;*

*(5) Any other laboratory divisions, sections, or functions which, in the opinion of the board, may serve the needs of law enforcement in the State of Arkansas for laboratory analysis.*

*History. Acts 1979, No. 864, §§7, 8; A.S.A. 1947, §§42-1209, 42-1210.*

### 1.2: Quality Manual

The section quality manual is a compilation of policies and procedures for use in the Forensic Toxicology Section in compliance with Arkansas State Statutes.

This quality manual is used by the analysts of the Forensic Toxicology Section and is reviewed annually and is updated as needed to reflect changing organizational, technical and procedural information. Changes to this quality manual may be initiated by any employee of the Forensic Toxicology Section upon preparing the information for presentation to the Chief Forensic Toxicologist, who will then gain the necessary approval(s) from the Scientific Operations Director and Executive

Director. The Quality Assurance Manager will be responsible for controlling this document. The controlled version of this manual shall be a PDF document stored on the ASCL computer system. Copies (printed or electronic) are uncontrolled versions. The Chief Forensic Toxicologist will be responsible for informing employees of new versions of the quality manuals.

It is recognized that unforeseen circumstances may arise which require immediate deviations from the policies and procedures of this manual. In such situations, the request for exceptions to policy affecting many cases will be submitted in writing to the Chief Forensic Toxicologist, or designee, and the request must include an adequate description of the circumstances requiring the action, a statement of the proposed alternative policy and procedure, and the intended duration of the exception. The Chief Forensic Toxicologist will maintain documentation of the approved policy exception. Deviations which only affect a small number of cases may be documented in the case file(s) without the aforementioned requirements.

COPY

## **Section 2: Personnel Qualifications and Training**

### ***2.1: Minimum Qualifications***

The minimum qualifications for the position of Forensic Toxicologist are:

- The formal education equivalent of a bachelor's degree in chemistry, biology, or a related field
- Knowledge of the principles of chemistry and chemical analysis

The minimum qualifications for the position of Chief Forensic Toxicologist are:

- The formal education equivalent of a bachelor's degree in chemistry, biology, or a related field
- Five years experience in a chemical laboratory including two years as a forensic toxicologist
- One year in a leadership capacity
- A master's degree can be substituted for all or part of these basic requirements upon approval of the Executive Director and the Scientific Operations Director.

### ***2.2: Job Description***

Examples of the work of a Forensic Toxicologist include:

- The analysis of biological samples for the presence and levels of alcohol, drugs, and other toxic substances
- The documentation of these analyses for future reference
- The generation of a report of laboratory analysis based on the analyses conducted
- Testifying as an expert witness as needed regarding the results of laboratory analysis
- Providing technical assistance to outside agencies on sample handling and general information on toxicological analysis
- Routine and preventative maintenance on laboratory equipment
- Attending staff meetings, professional meetings, and seminars
- The development and validation of new analytical procedures
- Technical and administrative review of case files
- Other duties as assigned

Examples of the work of the Chief Forensic Toxicologist include those above, as well as:

- The supervision of a staff of Forensic Toxicologists
- The preparation and initial approval of purchase requisitions

### ***2.3: Training***

All employees of the Forensic Toxicology Section must be trained before assuming casework duties.

This training is specified in the Section Training Manual and includes, but is not limited to:

- Introduction to the laboratory facility
- Organizational structure and chain of command
- Legal aspects of working in a forensic laboratory (e.g. security, confidentiality)
- Instrumentation theory and practice
- Health and safety
- Evidence handling
- Standard analytical methods
- Quality control
- Report writing
- Competency testing

## Section 3: Facilities and Security

### 3.1: Section Facilities

The Forensic Toxicology Section is located in the northeast corner of the third floor of the main building of the Arkansas State Crime Laboratory complex. It consists of four main areas, as listed:

- Office area (Rooms 323 and 324)
- Instrument bay (Room 331) – houses the gas chromatographs (GCs), gas chromatograph-mass spectrometers (GC-MSs), liquid chromatograph-mass spectrometer (LC-MS), and related equipment
- Extraction area (Rooms 328 and 329) – houses benches, two vent hoods, walk-in refrigerator (identified as Room 325), ELISA, UV-Visible spectrometer, SPE manifold, evaporator, and freezer
- Extraction area (Room 327) – houses benches, two vent hoods, AxSYM, CO-Oximeter, and refrigerated chemical storage.

In addition, the Toxicology Section houses the glassware cleaning room, identified as Room 326, although it is not a part of the Toxicology Section.

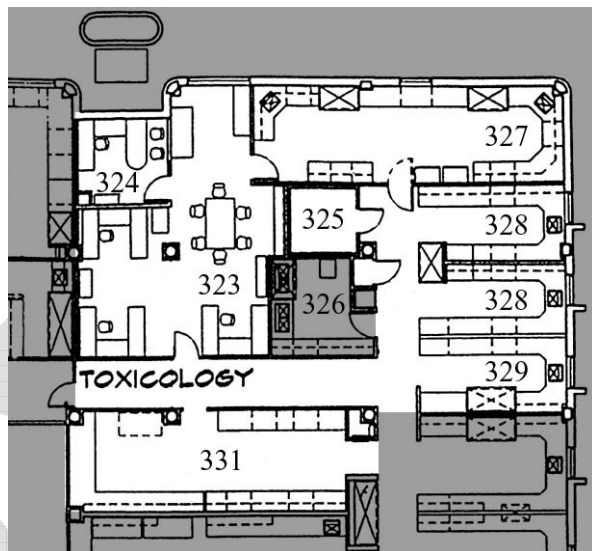


Figure 1 - Forensic Toxicology Section Floorplan

### 3.2: Section Security

The Forensic Toxicology, Illicit Labs, and Forensic Chemistry Sections are accessible to members of the Forensic Toxicology, Forensic Chemistry, Illicit Labs, and Trace Evidence Sections due to the physical layout of the laboratory. Access to the Forensic Toxicology Section is restricted at all times to authorized personnel, which includes members of the above-listed sections. This is accomplished by means of locked doors which prevent access from the central hallways without a security access card. Because the lab area is secured at all times by magnetic locks requiring a security access card for access, the doors inside the Forensic Toxicology section need not be locked either during working hours or after working hours. All refrigerated specimen storage is a common storage area which is locked when not in use or under direct observation.

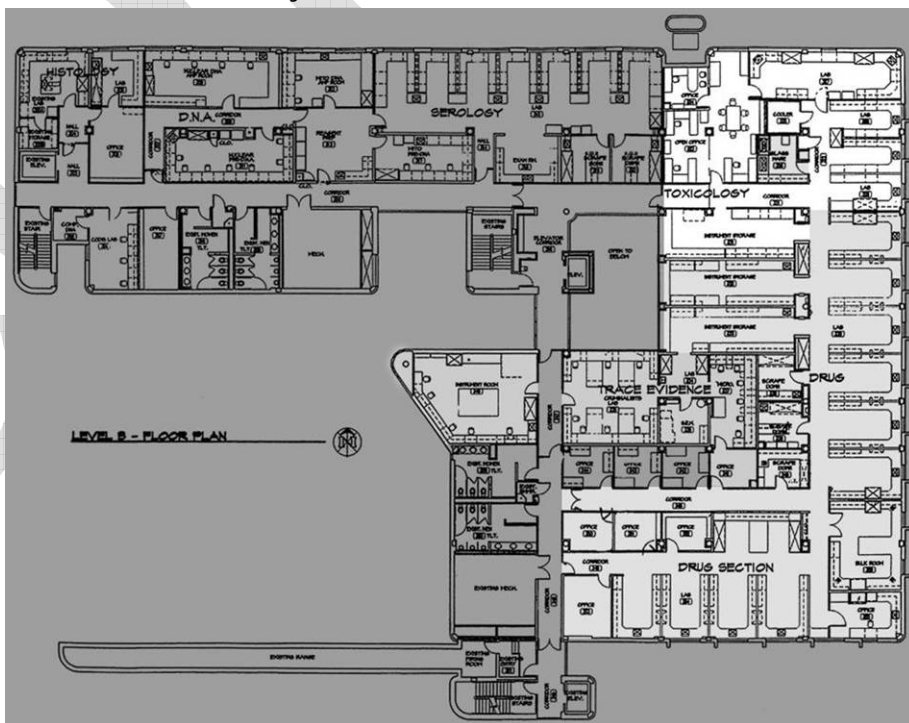


Figure 2 - Third Floor Floorplan

## **Section 4: Evidence Control and Case Management**

### ***4.1: Introduction***

The disposition of all evidence in the Arkansas State Crime Laboratory is recorded by a chain of custody. This chain of custody is primarily electronic, but may have written components which are stored in the appropriate case record. The chain of custody records the following information for each transfer of evidence:

- The date and time of the transfer
- The person/location/disposition from which the evidence is being transferred
- The person/location/disposition to which the evidence is being transferred
- An indication of the verification of the security of the transfer, which may be a signature on a written chain of custody, or an indication of verification by password if on an electronic chain of custody

The LIMS (Laboratory Information Management System) program is normally used to track all transfers of evidence between analysts and other personnel or storage locations.

### ***4.2: Accession of evidence***

Evidence submitted for toxicological analysis is submitted by an outside agency or by the medical examiner's office. Evidence is accessed into the Forensic Toxicology Section in one of two ways: it is brought directly to the Forensic Toxicology Section from the Medical Examiner's Office, or it is received from the Evidence Receiving Section.

When evidence is brought directly to the Forensic Toxicology Section from the medical examiner's office, it must be accepted by a Forensic Toxicologist or other person allowed to transfer evidence within the Forensic Toxicology Section. The process is as follows:

1. The specimens are brought to the Forensic Toxicology Section
2. The specimens are transferred from the submitter to the Forensic Toxicology Section secure storage via an analyst using the LIMS
3. Each item of evidence is sealed with tape if initially unsealed
4. The specimens are placed in refrigerated storage

When evidence is initially accepted and stored by the Evidence Receiving Section the following process occurs:

1. An Evidence Receiving Technician retrieves the appropriate specimens from secure storage
2. The specimens are transferred from the Evidence Receiving Technician to a Forensic Toxicologist using the LIMS
3. The specimens are transported to the Forensic Toxicology Section and stored in refrigerated storage
4. Upon opening the exterior packaging, each unsealed item of evidence must be sealed with tape if analysis is not started immediately

### ***4.3: Storage***

Evidence is stored in refrigerated storage inside a locked walk-in refrigerator or inside a locked freezer while awaiting analysis. These common storage areas are available only to the members of the Forensic Toxicology Section and other authorized personnel. All evidence in storage must be maintained in a sealed state unless it is in the process of examination, during which time it may remain unsealed. Once a case is completed, all unsealed items of evidence from that case must be resealed.



The medical examiner can ask that specimens be retained indefinitely for a particular case. Specimens will also be retained indefinitely upon court order. A \$25.00 fee for every six months of storage may be assessed for requests without a court order from outside the agency. Specimens may also be transferred to the submitting agency for long term storage.

#### ***4.4: Case Priority Policy***

Cases undergo initial testing in batches shortly after receipt, usually consisting of volatiles and/or immunoassay testing. Cases requiring further analysis are analyzed in chronological order as needed. Rush cases may be analyzed out of chronological order. They may be assigned by any Forensic Toxicologist but must be assigned as soon as practicable after the case files are taken into possession by the analyst. Stat priority cases are assigned to any analyst that can perform the analyses quickly.

#### ***4.5: Disposal***

As cases are completed the specimens associated with these completed cases are resealed with tape and placed in storage boxes to await disposal. Homicide cases are stored separately from other cases. The procedure for boxing these samples is as follows:

1. Transfer the case from the possession of the analyst or the Forensic Toxicology Section to the possession of a named box. This information will be recorded in the chain of custody, normally using a batch transfer in the LIMS.
2. When the box is filled, the box is sealed and the date that the last case was put in the box is recorded. The date of the last transfer for items in the box is also available in the LIMS database.

After six months, the specimens from non-homicide cases may be destroyed if no request for retention or further testing has been received. Homicide cases are stored indefinitely and are not routinely destroyed. A record of all specimens destroyed will be maintained, listing the identity of each specimen, the date it was destroyed, and the identity of the person destroying the specimen. Specimens are disposed of as biomedical waste to an approved contractor or in another accepted manner.

#### ***4.6: Release***

Specimens can be released to the submitting agency if requested by that agency. Specimens submitted by the medical examiner's office may be returned to them directly using the internal chain of custody system. Release to anyone other than, or by the direction of, the submitting agency requires a court order. All releases to outside agencies will be documented in the case record.

Specimens sent for outside testing may be retained and/or destroyed by the outside testing agency. These specimens are not considered released by the laboratory, but rather consumed as a result of analysis.

#### ***4.7: Cessation of Analysis***

The Forensic Toxicology Section may discontinue further forensic examinations when the toxicological results support the maximum charge to be filed.

#### ***4.8: Cases Inappropriate for Analysis***

Certain types of cases are inappropriate for analysis and will not routinely be analyzed. Examples include, but are not limited to:

- Law enforcement cases where the toxicological analysis is not probative to criminal charges, such as:

- Cases where toxicology results are being requested to attempt associate a subject with the possession or manufacture of a controlled substance
  - Testing of a third party to a crime (such as a passenger in a DWI vehicle)
  - Cases submitted for informational purposes only, such as cases with no charges
- Cases where the sample submitted is unsuitable for testing due to type or amount
- Cases where the specimens have leaked from the container or may otherwise have been subject to contamination
- Evidence items not consisting of biological specimens, such as possibly adulterated food or drink

#### ***4.9: Evidence Marking and Sealing***

All evidence will be marked or identified with the unique laboratory case number, if practical (e.g. YYYY-##### or YYYY-#####). Otherwise the proximal container must be marked or identified with the unique laboratory case number. Each exterior container must have its appropriate barcode label affixed to it.

Evidence will be sealed in a manner in which the contents cannot readily escape and in such a manner that opening the container would result in obvious damage or alteration to the container or its tape seal. All evidence must bear a proper seal which shall include the initials or other identification of the person sealing the evidence across the seal.

#### ***4.10: Inter-Laboratory Evidence Transfer***

The Forensic Toxicology Section policies regarding inter-laboratory evidence transfer conform to the lab-wide policies and may be found in the lab-wide quality manual.

## **Section 5:      Validation**

The Forensic Toxicology Section policies regarding validation conform to the lab-wide policies and may be found in the lab-wide quality manual.

COPY

## Section 6: Analytical Procedures

### 6.1: Introduction

Standard analytical procedures are important in ensuring quality. The following standard operating procedures help to ensure that the analysis of each case is done in a manner consistent with scientific principles and the needs of the case. Any significant deviation from the standard operating procedure must be documented in the case file.

### 6.2: General requirements

Cases should routinely be analyzed in chronological order. Exceptions to this guideline may be made in response to:

- Properly documented Medical Examiner request for stat blood alcohol and/or carboxyhemoglobin (COHb) levels
- Requirements of the Office of the Medical Examiner (e.g. for NAME accreditation)
- Priority requests
- Convenience of analysis (e.g. analyzing samples in batches for reasons of economy of scale)

The amount of specimen available in a given case should be considered when determining a course of analysis. If the specimen amount is insufficient to perform all of the analyses requested, the analyst should attempt to prioritize the requests for analysis based upon the information obtained from the submission sheet. If there is insufficient information available to prioritize the requests then it is advisable to contact the submitting agency for guidance.

It is permitted to analyze a smaller sample amount than normal if necessary, but a disclaimer should accompany any negative findings indicating that insufficient sample was available for normal testing.

The normal amount of specimen requested for various assays are:

Blood alcohol	5-10 mL blood
Blood drug screening – qualitative only	10-15 mL blood, preferably with preservative
Quantitation (each drug)	10 mL blood
Carbon monoxide	5 mL blood
Urine THC	5 mL urine
Opiates	10 mL blood
Arsenic/heavy metals	30 mL blood or 10 g tissue
AxSYM FPIA screening	5 mL urine
ELISA screening	5 mL blood or urine
Blood immunoassay screening	5 mL blood

Other specimen types may be appropriate depending on the circumstances of the case.

### 6.3: Course of analysis

#### 6.3.1: Initial Screening

An initial screening is routinely performed on cases as they are assessed into the Forensic Toxicology Section. This screening consists of a blood alcohol and immunoassay test, as appropriate. Other testing may be performed at this point if required. If more analysis is required after this screening, the file is distributed to a qualified Forensic Toxicologist for analysis.

## 6.3.2: Law enforcement cases

### 6.3.2.1: DUI cases

The normal progression of analysis for a driving under the influence/driving while intoxicated (DUI/DWI) case is as follows:

1. Initial screening
  - Blood alcohol analysis (if appropriate)
  - Immunoassay screening (if appropriate)
2. Acid/base extraction (if appropriate)
3. Generation of the Report of Laboratory Analysis

DUI/DWI cases are generally submitted to determine a cause for the impairment of an individual. In DUI/DWI cases where both alcohol and drug screens are requested, the blood alcohol analysis should be performed first. If the subject is above the applicable *per se* ethanol value, then no further analysis is required unless the case is associated with a Drug Recognition Expert (DRE) conclusion. In these cases an immunoassay or qualitative drug screen is performed, if the sample size permits. Blood is the preferred specimen, if available in sufficient quantity. The submission sheet should reflect that the law enforcement officer has performed a DRE evaluation and the class of intoxicant should be indicated.

*Per se* ethanol values vary. For subjects under twenty-one years of age, the *per se* ethanol limit is 0.02 g% (A.C.C. § 5-65-303). For commercial drivers or aircraft operators, the *per se* ethanol limit is 0.04 g% (A.C.C. § 27-23-114). For other drivers, the *per se* ethanol limit is 0.08 g% (A.C.C. § 5-65-203). At or above these values, the subject is considered legally intoxicated, and further analysis is unnecessary to demonstrate intoxication.

Ethanol values greater than 0.04 g% may be used in court to explain observed impairment. Therefore, if impairment is noted in the case record and the ethanol value is 0.05 g% or greater, then no further initial analysis is required.

Urine alcohol testing is not performed unless the urine was sampled according to guidelines promulgated by the Arkansas Department of Health Office of Alcohol Testing, which include an initial voiding of the bladder, a thirty minute waiting period, and a second voiding of the bladder for urine collection.

If urine is available, a urine immunoassay screen should be performed. If only blood is available, an ELISA immunoassay should be performed if the alcohol level is not sufficient to discontinue analysis.

In cases where the blood alcohol results and immunoassay screen are negative but impairment was indicated by the officer, a qualitative base screen is performed. Blood is the preferred specimen, if available in sufficient quantity. Efforts should be made to keep one milliliter of specimen in reserve, if possible, for further testing (such as the differentiation of methamphetamine and phentermine).

Additional testing may be required in some cases. The course of additional testing depends on the details of the case.

### 6.3.2.2: Other cases

The course of analysis for other law enforcement cases is dependant upon the needs of each individual case. A course of analysis should be determined based upon the request(s) of the law enforcement agency, our capabilities, and the needs of the case.

Aircraft crashes are under the jurisdiction of the FAA and/or NTSB and no toxicological samples from these cases are tested.

#### **6.3.2.3: ME cases**

The progression of analysis for medical examiner and coroner cases is as follows:

1. Stat alcohol and/or COHb analysis (if requested)
2. Initial screening
  - Immunoassay screening (if requested)
  - Blood alcohol (if requested)
3. COHb analysis (if requested)
4. Blood drug screening (acid and base fractions, if requested)
5. Further testing (if requested)
  - Confirmation (if requested)
  - Quantitation (if requested)
6. Generation of the Report of Laboratory Analysis

In many cases less analysis is necessary, and in these cases testing is limited to that requested by the medical examiner's or coroner's office.

Some pathologists request that more specimen types be analyzed on all cases with a positive blood ethanol. If requested, urine, bile, vitreous, and gastric contents will be analyzed for ethanol.

Blood acid and base screens should be run on the GC-MS and, at a minimum, all positive specimens should be analyzed by GC for comparison against historical quantitation curves. Efforts should be made to keep one milliliter of specimen in reserve, if possible, for further testing.

Certain ubiquitous drugs such as caffeine and nicotine are not reported or quantitated unless present in uncharacteristically large amounts. Lidocaine and atropine are generally not quantitated, but are reported.

Other testing may be necessary based on the needs of each case.

### ***6.4: Acceptable Work Product***

#### **6.4.1: General Requirements**

The following sections define what makes up an acceptable work product. If one or more of these criteria cannot be met, the results from that assay may not be reported out without the written approval of the Chief Forensic Toxicologist or their designee.

All dilutions of certified standards will be made using measured volumetric amounts.

When assays are performed by an analyst other than the one signing the report, the analyst doing the assay will initial all results generated. The analyst who signs the report will also need to indicate by initialing that they have reviewed this data. This may be recorded on the Results Worksheet (TOX-FORM-03) for results appearing on that worksheet, or by initialing each page of work performed by another analyst. In either case, the initials indicate that the analyst who signs the report agrees with the analytical results for all analyses.

Any urine screens should be qualitative only and any positive results are reported "*present*". Negative results are reported as "*not detected*".

Exceptions to some of these guidelines may be approved on a case-by-case basis by the section chief and/or quality manager. Written approval of the exception including a justification of the variance will appear in the case file.

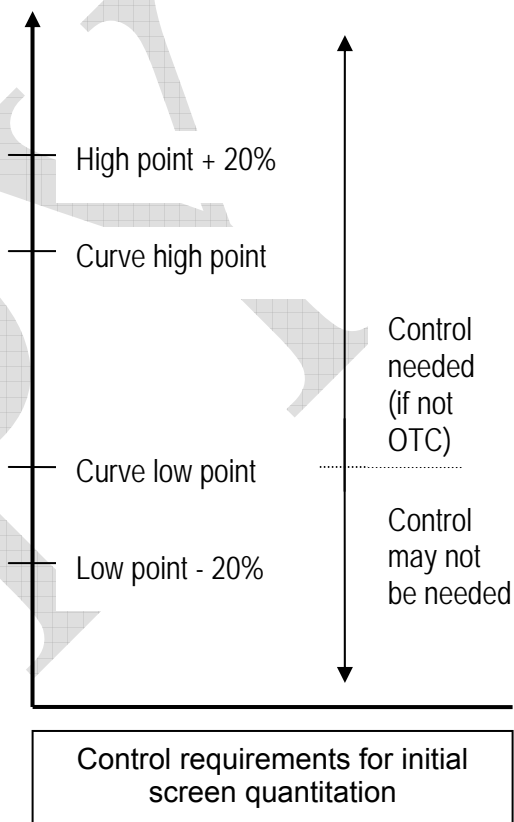
#### 6.4.2: Controls

Positive and negative controls are analyzed whenever possible. If the measured values of a control differs more than the accepted amount for a quantitative assay, further investigation to determine the source of the discrepancy and appropriate action to correct it is warranted. This normally requires the extraction and analysis of a new control. This control may be from the same source to help determine if the problem was in the extraction, or from a third source to help determine if the issue is with the control sample. If it is demonstrated that the curve is in error then a new calibration curve must be constructed. If it is demonstrated that the error is confined to the original control sample(s) then the cases may be quantitated against the curve. If a problem in the extraction is demonstrated, then re-extraction of the samples is necessary.

If a positive or negative control does not behave in the expected manner in a qualitative assay, another control is reanalyzed using the same method. This control may be from the same source to help determine if the problem was in the preparation, or from a third source to help determine if the issue is with the control sample.

Certified standards should be used whenever possible. If no certified standards are available, then an uncertified primary standard (such as a bulk powder or liquid from a chemical supplier) may be used, providing its quality has been verified. If no uncertified primary standard is available, then tablets or capsules of stated content may be used.

In a quantitative assay, a control sample will be run if drugs are present in quantities that fall on the appropriate analytical curve. Over-the-counter (OTC) drugs are exempt from this requirement, unless a possibly harmful amount is indicated to be present. If the drugs are indicated to be present in quantities below the limits of the analytical curve, then no control is needed unless it is reasonable that the amount present might be toxic or lethal.



#### 6.4.3: Quantitations

The correlation coefficient of an analytical curve must exceed 0.990 and the measured value of no curve point may vary more than 20% from the known value of that curve point, with the exception of the lowest point on the curve, which may vary up to 30% from its known value (as may a control made at this concentration).

#### 6.4.4: Notes

The listed standard abbreviations will be used for common specimen types. Other abbreviations may be used to differentiate types not listed, to accommodate multiple specimens of the same type, or to differentiate between different subjects in the same case. The abbreviation listed on the accession sheet and associated uniquely with the specimen identifier must appear on each page of analytical data generated by the analysis of that specimen in order to correlate the analytical data to the correct specimen. If specimens are combined for analysis, then a new abbreviation should be made and a description of the composition of this new specimen must appear in the case record.

For homogenates and other dilutions, the dilution factor as well as a short description of the preparation must be included in the case notes.

<i>Specimen</i>	<i>Abbreviation</i>
AM Blood	AB
Heart blood	HB
Peripheral blood	PB
Stat blood	SB
Unknown blood	UB
Urine	UR
Vitreous	VT
Gastric	GS
Bile	BL
Pericardial fluid	PR
CSF	CS
Subdural	SD
Cavity fluid	CV
Liver	LV
Kidney	KD
Muscle	MS
Lung	LN
Brain	BR
Pleural fluid	PL

#### 6.4.5: Additional aliquots

If the initial quantitative result is possibly elevated, then the initial quantitative result should be confirmed whenever possible by a second quantitation of the same specimen. 'Possibly elevated' is defined as a level which is consistent with a concentration associated in the literature with a toxic or lethal level, or is inconsistent with a concentration associated in the literature with a therapeutic level, or a case where there is no information concerning these levels.

#### 6.4.6: Additional Assays

The presence of drugs in a specimen should be confirmed, if possible, with a second analytical technique based on a different principle. For example, the presence of methamphetamine in a base screen could be confirmed by a positive immunoassay result for the amphetamines class. The presence of meaningful drugs in a specimen should be confirmed, if possible, with a second specimen or a second aliquot of the same specimen. This helps rule out contamination during extraction or transitory instrumental contamination.

#### 6.4.7: Contamination countermeasures

The prevention of contamination is of primary importance in a toxicology laboratory. There are many things that are done to both prevent and detect contamination, among them:

- Use of disposable glassware and other consumables whenever possible
- Analysis of duplicate samples
- Looking for common results in a batch
- Looking for the absence of appropriate levels of metabolites
- Washing items from the Forensic Chemistry and Forensic Toxicology Sections separately
- Solvent-rinsing of any cleaned, reused glassware



## **6.4.8: Method specific requirements**

### **6.4.8.1: Gas Chromatography**

In methods used to detect and identify an analyte, the signal-to-noise (S:N) ratio for a peak must be at least 10:1. The S:N ratio for a blank must not exceed 3:1 within a retention time window of  $\pm 2\%$  around the peak of interest.

In methods used to quantitate an analyte that has already been identified using a different method, the signal-to-noise (S:N) ratio for a peak must be at least 10:1. The S:N ratio for a blank must be less than 3:1, or less than 2% of the area of the peak for which it is the blank, within a retention time window of  $\pm 2\%$  around the peak of interest.

The retention time of an analyte should not differ more than  $\pm 2\%$  from the retention time of its control.

If a column is clipped or a new column is installed and this affects the retention time of an analyte, the retention time of that analyte in a calibrated method may be changed without recalibration of the method if a known standard of that analyte is run to determine the new retention time.

Columns of different phases, phase ratios, and length may be substituted for the listed columns in gas chromatographic methods providing that positive and negative controls are run and perform adequately.

Internal standards may be changed if needed to help with co-eluting peaks or other analytical difficulties.

Instrumental conditions may be temporarily changed to assist in the analysis of a particular analyte, but the instrumental conditions will remain standardized for each method. Any changes must be documented in the case record.

### **6.4.8.2: Gas-Chromatography Mass-Spectrometry (GC-MS)**

In a method using selected ion monitoring (SIM), the ratio of the qualifier ion(s) relative to the quantitation ion should not differ more than 20%, or the amount specified in the method. At least one SIM qualifier ion must be present.

The signal-to-noise (S:N) ratio for a peak must be at least 10:1. The S:N ratio for a blank must not exceed 3:1 within a retention time window of  $\pm 2\%$  around the peak of interest. S:N ratios may be evaluated by using the total ion chromatogram, an extracted ion chromatogram using ions characteristic of the analyte in question, or the selected ion chromatogram. In a GC-MS SIM-mode method, the failure of qualifier ion ratios is sufficient to consider a negative control blank.

A scan-mode mass spectral identification must be based upon a match to a control, library, literature, or otherwise known spectrum. All significant peaks (generally above 10% of the base peak) in the known spectrum should be in the unknown spectrum, or their absence must be explainable. All other major peaks must be explainable.

#### 6.4.8.3: Carboximetry

Using a CO-Oximeter or UV/Visible spectrometer, a matrix blank cannot contain more than 5% carboxyhemoglobin saturation. The positive control must be within the range reported by the manufacturer. If either control is out of range, it may be rerun.

Using Conway diffusion, the positive control must be clearly more positive than the negative control. The negative control must not have any metallic film.

#### 6.4.8.4: AxSYM

Any instrumental exceptions must be investigated and corrected if possible. If the exception in that assay cannot be corrected, then the results for that assay and specimen cannot be reported.

#### 6.4.8.5: ELISA

Absorbance must decrease with increasing concentration of analyte.

There is no minimum correlation coefficient of any analytical curve obtained due to the inherent nonlinearity of the method.

#### 6.4.8.6: Reinsch

The positive control must have clearly more dull deposit than the negative control, which should have no deposit at all.

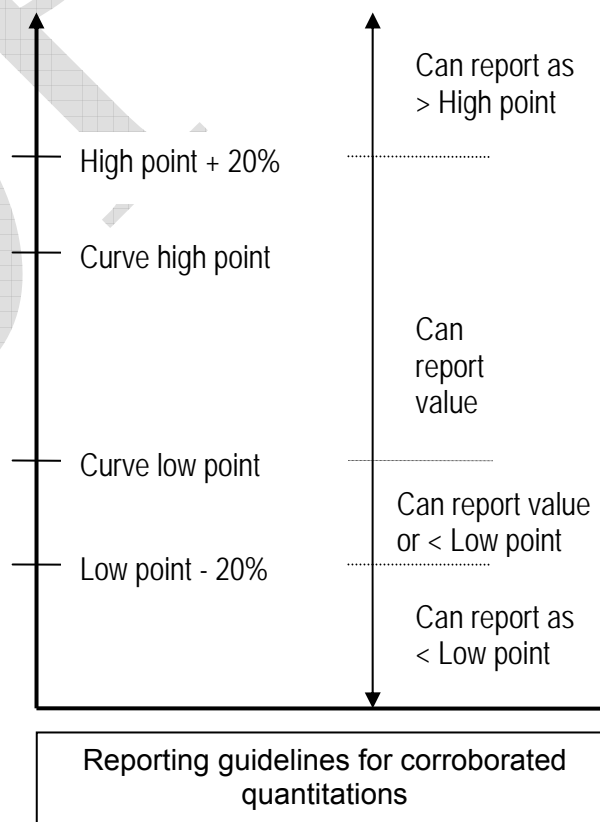
### 6.5: Reporting

#### 6.5.1: General requirements

Analytical results must be clearly associated with the specimen(s) from which they are derived.

The initial results of a stat carboxyhemoglobin (COHb) assay may be reported to the requesting pathologist after the completion of any one test for carboxyhemoglobin. Positive results must be confirmed by a second method if the case history is inconsistent with the obtained result.

If the results of a quantitation are positive but fall more than 20% below the value of the lowest calibration standard then one of three courses of action may be appropriate. The results may be reported as "positive, but less than" the value of lowest calibration standard, adjusted for the amount of specimen extracted (if necessary). A new calibration standard may be extracted to bracket the quantitation result. The sample may be re-extracted using a larger aliquot to ensure that the result lies within the range of the calibration standards.



If the results of the quantitation fall more than 20% above the value of the highest calibration standard then one of three courses of action may be appropriate. The results may be reported as

“greater than” the value of the highest calibration standard, adjusted for the amount of specimen extracted (if necessary). A new calibration standard may be extracted to bracket the quantitation result. The sample may be re-extracted using a smaller aliquot or a dilution to ensure that the result lies within the range of the calibration standards.

Immunoassay results should not be reported if they have been confirmed by other analyses unless they have been previously reported in a report of preliminary testing, with the exception of negative opiates and cannabinoids results (and barbiturate results if an acid screen is not performed). These opiates and cannabinoids results are not confirmed by standard acid and base screens and therefore these negative results may be instructive. Immunoassay results not confirmed by additional testing should not be listed on the report.

### 6.5.2: Language

Immunoassay results should be reported as “positive” or “negative”.

If the results of a general screen are negative this may be reported as “No drugs detected”.

If the results of a screen for a particular analyte are negative this may be reported as “Not detected” unless the assay(s) used would not normally detect the specifically targeted analyte, in which case this should be stated on the report.

If drugs are detected in an acid or base screen but not detected in a second aliquot, then those results should not be reported.

Although the number of reported significant figures is typically two, this is a matter of professional judgment and is at the discretion of the analyst.

#### 6.5.2.1: Disclaimers

A disclaimer may be necessary to clearly define the meaning and limitations of toxicological testing. The following is a list of situations where a disclaimer may be appropriate and a standard disclaimer that may be used. Other situations may require different disclaimers. A disclaimer must be used when appropriate.

Situation	Disclaimer
Initial quantitative values are uncorroborated for any reason	<i>The reported drug amount has not been corroborated by replicate analysis.</i>
No certified standards are available upon which to base the results of an assay.	<i>No certified standard was available for this analyte. The reported drug amount is based upon a standard of uncertified purity.</i>
Other incomplete testing due to lack of sufficient sample.	<i>Insufficient sample is available for normal testing.</i>

All reported immunoassays without further confirmation will include the disclaimer:

*Note: Screening of the specimen(s) submitted has yielded the following preliminary results. Should confirmatory or additional testing be required, you must contact this office within ninety (90) days of the issuance of this report. The specimen(s) will be destroyed after ninety (90) days.*

## **6.6: Standards and Reagents**

### **6.6.1: General requirements**

All analytical standards must be verified prior to use. A certificate of analysis will suffice for verification. All records of verification must be kept on file. The standard shelf life for a solid drug standard is five (5) years. Any standard may be used after its expiration date by a reverification of its quality by mass spectrometry or infrared spectroscopy. This reverification will suffice for one (1) year, after which the standard must be reverified again. Any prepared standard used for quantitation may be used after its expiration date by a reverification of its quantitative amount by comparison against a historical curve. This reverification will suffice for one (1) year, after which the standard must be reverified again.

Chemicals and solvents transferred to a different container for use in the work area (e.g. ammonium hydroxide) need only be labeled with the identity of the chemical or solvent and the lot number of the original source.

The standards used in each assay must be identified in the case file. This is accomplished through the use of a standard number which is assigned to each standard as it is made. This allows the tracking of all standards back to their original source. If a dilution is made of the standard before addition to a specimen, the dilution factor must be noted in the case file.

### **6.6.2: Standards/controls**

All controls must be logged in a logbook with the following information:

- Source
- Lot number, when available
- Date received/prepared
- Control identification number
- Expiration date, if appropriate
- Quality control results, if appropriate

When a new standard is received, the following procedure should be followed:

1. Mark the container with the date the standard was received and the initials of the analyst that received it.
2. Add an entry in the standard verification log (TOX-FORM-07) listing the salt form/molecular weight of the material, the vendor, lot number, date of receipt, and stated purity, if known. If a certificate of analysis is provided, this should be noted as the verification method.
3. Before analytical results based on a standard are released, the standard must be verified. A Certificate of Analysis suffices for verification. If no Certificate of Analysis is available, an unextracted sample of the standard should be analyzed using GC-MS or FTIR to ensure that the composition of the standard is consistent with the stated purity.
4. A reagent may be verified by use in an assay if run for comparison with the same reagent which was previously verified, preferably in a control.

When a new standard solution is prepared, the following procedure should be followed:

1. Add an entry to the standard preparation log (TOX-FORM-06) listing the drug name, vendor, preparer, lot number, date of preparation, the solvent used, the final concentration of the standard, an expiration date (generally one year) and a description of the preparation of the standard.

2. A standard number is assigned to the prepared standard. This number is generally assigned sequentially, with a prefix of C for certified standards and U for uncertified standards (e.g. C-16, U-571)
3. When the standard is used to make or check a calibration curve, the results of this analysis should be recorded in the standard preparation log. If the calibration curve and the control samples agree, this is recorded as passing. If they do not agree, and it is determined that the newly generated standard solution is in error, this is recorded as failing.

### **6.6.3: Reagents**

#### **6.6.3.1: Purchased**

All purchased reagents must be labeled with the following:

- Lot number
- Date opened
- Expiration date, if applicable
- The initials of the analyst who opened the container
- Date received and initials

#### **6.6.3.2: Prepared**

All prepared reagents must be labeled with the following:

- Reagent name
- Preparation date
- Expiration date

A logbook must be kept for all prepared reagents, which must include the following:

- Reagent name
- Preparation date
- Expiration date
- Reagent preparation instructions
- Lot numbers of solvents/chemicals used to prepare reagent
- A method to verify the reliability of the reagent, if applicable
- Initials of the person verifying reagent reliability, if applicable

## **6.7: Standard Operating Procedures – Analytical Methods**

### **6.7.1: AxSYM Screen**

#### **Principle**

The AxSYM is a fluorescence polarization immunoassay (FPIA) technique based on competitive binding to an antibody between the analyte and a fluorescent tracer. The more analyte there is in a specimen, the less fluorescent tracer can bind to the antibody to form a fluorescent complex that can be detected by the AxSYM.

#### **Specimens**

This method is applicable to urine. The specimen size is determined by the number of analytes chosen for analysis.

#### **Reagents**

AxSYM reagent packs  
AxSYM solution 4 (Diluent)

#### **Controls**

AxSYM controls  
AxSYM calibrators

#### **Equipment**

Micropipetter  
Reaction vessels  
Sample vials

#### **Instrumentation**

Abbott AxSYM

#### **Instrument Conditions**

Instrument conditions are set by the manufacturer.

#### **Procedure**

1. Perform any daily, weekly or monthly maintenance required by the instrument operating manual
2. Run controls to ensure the instrument is functioning properly
3. File control results
4. Run samples to be analyzed in accordance with the manufacturer's instructions making sure that each sample is properly identified with its ASCL case number
5. Upon completion of analysis, place the reagent kits and samples back in refrigerated storage
6. Ensure that the AxSYM scans the empty tray to turn off its internal reagent time clock
7. Place results in appropriate case files

#### **Quality Assurance**

The Abbott AxSYM determines whether controls are acceptable. A Levey-Jennings plot of control results is available.

A negative control consisting of AxSYM solution 4 (a buffer) is to be run with the other controls as an instrument blank.

#### **Precautions and Notes**

The results of an immunoassay screen are reported as "*positive*" or "*negative*".

Any instrumental exceptions must be investigated and corrected if possible. If the exception in that assay cannot be corrected, then the results for that assay and specimen cannot be reported.

**Preparation of Materials**

Materials are purchased fully prepared.

COPY

## 6.7.2: Ethanol Analysis

### Principle

This method is designed to detect the presence of ethanol and other volatiles in blood, urine, vitreous, gastric, tissue homogenate, and other biological fluids by headspace gas chromatography using dual column analysis. Ethanol is identified by relative retention time.

### Specimens

This procedure is appropriate for blood, bile, urine, gastric, vitreous, other body fluids, and homogenized tissue or clot samples. A sample size of 0.1 mL or 0.2 g of a 1:1 tissue homogenate is generally used.

### Chemicals and Reagents

0.05% n-propyl alcohol v/v in deionized water

Methanol

Isopropanol

Acetone

### Controls

Certified ethanol controls (purchased, concentration may vary)

Calibration curve ethanol standards: 0.010, 0.020, 0.050, 0.100, 0.200, 0.300, 0.400 g%

Calibration curve methanol, acetone, isopropanol standards: 0.010, 0.020, 0.050, 0.100, 0.200 and 0.400 g% (each)

Methanol, acetone, and isopropanol control sample

### Equipment

20 mL sample vials designed to accommodate 20 mm crimp-on rubber septa

Volumetric pipetors for the range of 100 µL through 1000 µL

Crimper

### Instrumentation

Gas chromatograph:	HP 6890 or equivalent
Headspace unit:	PE HS40XL or equivalent

Column type:	Rtx-BAC1	Rtx-BAC2
Length (m):	30	30
ID (mm):	0.32	0.32
Film thickness (µm):	1.8	1.2

### Instrument conditions

Inlet	
Mode:	Manual
Inlet temp (°C):	100
Purge:	Off
Column	
Mode:	Constant pressure
Pressure (psi):	10.00
Initial flow (mL/min):	2.4
Avg. velocity (cm/sec):	81
Detector	
Detector:	FID

Headspace Unit	
HS Mode:	Constant
Oven temp (°C):	60
Needle temp (°C):	65
Transfer temp (°C):	80
GC cycle time (min):	8.3
Thermostat time (min):	12
Pressurization time (min):	0.5
Injection time (min):	0.04
Withdrawal time (min):	0.2
Vial venting:	On
Injection mode:	Time
Temperature zones:	Separately



Data rate (Hz):	20.000
Gas type:	Helium

Carrier gas pressure (psi):	27

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	50	5
30	95	0.5

### Procedure

1. Label sample vials appropriately for controls and the samples to be run
2. Pipette 1000 µL of 0.05% n-propyl alcohol into each vial
3. Pipette 100 µL of sample or control into the each previously labeled vials and cap each vial
4. Load vials into autosampler carousel, running controls to check agreement with the previously stored curve
5. Upon completion of the run, check for agreement between values and place the results in the proper case files

### Quality Assurance

A calibration curve is stored as part of the instrument method. A suggested calibration curve consists of the following data points (grams analyte/100 mL sample (g%)):

Analyte	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Ethanol	0.010	0.020	0.050	0.100	0.200	0.300	0.400
Methanol	0.010	0.020	0.050	0.100	0.200	0.300	0.400
Acetone	0.010	0.020	0.050	0.100	0.200	0.300	0.400
Isopropanol	0.010	0.020	0.050	0.100	0.200	0.300	0.400

At least two positive control samples must be run each day that samples are run. A control sample must be run for each analyte reported. The value of these controls will vary, but they must be within the curve. The measured values of all positive control samples must be within 10% of their known value or the range suggested by the manufacturer (if less strict). If a control sample falls outside that range another control sample is prepared and the control samples are rerun. If the control sample still fails, then further investigation and appropriate action is required before case samples are run.

A negative control consisting of blood bank blood must be analyzed in every batch. Any positive result requires further investigation and appropriate action.

It is recommended that alcohols be run in two batches. The first batch contains all of the samples to be analyzed. The second batch, which is aliquotted separately, contains only those samples which are positive for some analyte.

### Precautions and Notes

Other volatiles (including formaldehyde, toluene, and chloroform) can also be determined by this procedure using the proper controls and run times.

Decomposed specimens may yield greater variation between duplicate runs than is expected from fresh specimens, and should be followed in the sequence by a blank to prevent carryover.

Alcohol results may be reported if all aliquots of the same specimen give results within 0.005 g% or 10% (whichever is greater) from the average as calculated by the formula:

$$\text{Percent difference} = ((\text{Result} - \text{Average})/\text{Average}) \times 100$$

If there are values outside of the acceptable range, those samples may be reanalyzed. If there are instrumental reasons for values to be outside of the acceptable range (coeluting compounds, etc.) then these values may be discarded. The lowest of the results is reported, with results truncated to the second decimal place.

If methanol, acetone, or isopropanol are present then a control sample containing that compound must be run. Methanol, acetone, and isopropanol may then be reported if all aliquots of the same specimen give results within 0.005 g% or 10% (whichever is greater) from the average as calculated by the formula:

$$\text{Percent difference} = ((\text{Result} - \text{Average}) / \text{Average}) \times 100$$

If there are values outside of the acceptable range, those samples may be reanalyzed. If there are instrumental reasons for values to be outside of the acceptable range (coeluting compounds, etc.) then these values may be discarded. The lowest of the results is reported, with results truncated to the second decimal place. If methanol is present and the specimen was taken postmortem and may have been from an embalmed source, the following disclaimer must be added:

*Note: Methanol is a common component of embalming fluid.*

Positive results below the limit of quantitation should be reported "less than" that value. A positive result must show a peak in every run on both columns. Results greater than the highest point of the calibration curve should be reported as "greater than" that value.

## **Preparation of Materials**

### *Calibration curve standards:*

These standards are purchased in certified concentrations, used as supplied, except:

- The 0.010 g% level is prepared by aliquotting 20 µL of the 0.0500 g% standard and 80 µL of deionized water into the headspace vial in lieu of 100 µL of standard.
- The 0.020 g% level is prepared by aliquotting 40 µL of the 0.0500 g% standard and 60 µL of deionized water into the headspace vial in lieu of 100 µL of standard.
- The 0.200 g% level is prepared by aliquotting 50 µL of the 0.400 g% standard and 50 µL of deionized water into the headspace vial in lieu of 100 µL of standard.
- The 0.300 g% level is prepared by aliquotting 75 µL of the 0.400 g% standard and 25 µL of deionized water into the headspace vial in lieu of 100 µL of standard.

### *Methanol, acetone, isopropanol control sample (0.100 g%):*

Add 127 µL of methanol, 128 µL of isopropanol, and 127 µL acetone to a Class A 100 mL volumetric flask. Make up to 100 mL with deionized water.

### *0.05% n-propyl alcohol v/v in deionized water:*

Add 1 mL n-propyl alcohol to 2 L deionized water and mix well.

### 6.7.3: Base Screen

#### Principle

This method is designed to detect the presence of basic drugs by gas chromatography-mass spectrometry. The drugs are extracted from their biological matrix by liquid-liquid extraction and identified by their mass spectrum and relative retention time (if known). The instrument method is retention-time locked to methaqualone to allow for long-term stability of retention times and the use of a screener library.

#### Specimens

This method is applicable to urine, blood, serum, bile, tissue homogenates, and gastric contents. A 5 mL or 5 g sample is generally used unless circumstances warrant the use of a different sample size (e.g. very high or very low suspected drug levels).

#### Chemicals and Reagents

Concentrated ammonium hydroxide  
Concentrated hydrochloric acid  
1N Hydrochloric acid  
n-Butyl chloride (chromatographic grade)  
Hexane (chromatographic grade)  
Methanol (ACS grade)  
Chloroform (chromatographic grade)  
Water (reverse osmosis or Millipore)

#### Controls

Methaqualone stock: certified 1.0 mg/mL  
Methaqualone working solution (0.10 mg/mL)  
Base test mix: 0.1 mg/mL amphetamine, methamphetamine, diphenhydramine, amitriptyline, nortriptyline, oxycodone, and alprazolam

#### Equipment

15 mL screw cap centrifuge tubes  
Pipets and pipettors  
Tube rotators  
Centrifuge  
Aspirator  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper

#### Instrumentation

Gas chromatograph:	Agilent 6890 or equiv
Mass spectrometer:	Agilent 5973 or equiv
Autosampler:	Agilent 7683 or equiv

Column type:	ZB-5 or equiv
Length (m):	15
ID (mm):	0.25
Film thickness (µm):	0.25

#### Instrument conditions

Inlet	
Mode:	Pulsed splitless
Inlet temp (°C):	250
Pressure (psi):	10.0 (variable)

Column	
Mode:	Constant pressure
Pressure (psi):	10.00
Initial flow (mL/min):	2.4

Pulse pressure (psi):	20.0
Pulse time (min):	0.50
Purge flow (mL/min):	20.0
Purge time (min):	2.00
Total flow (mL/min):	25.2
Gas saver:	On
Saver flow (mL/min):	20.0
Saver time (min):	2.00
Gas type:	Helium

Avg. velocity (cm/sec):	81
Detector	
Detector:	MSD
Transfer line temp (°C):	280
Quad temp (°C):	150
Source temp (°C):	230
Mass range (amu):	35-550
Threshold:	150
Number of samples:	2
Solvent delay (min):	2.45 (variable)

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	100	0
15	280	10

## Procedure

1. Label the proper number of 15 mL extraction tubes for the samples and controls to be extracted
2. Pipette 5 mL of body fluid, control, blank, or 5 g 1:1 tissue homogenate (w/w in normal saline) into the labeled tubes
3. Adjust the pH of each specimen to approximately 9 by adding approximately 0.2 mL of concentrated ammonium hydroxide
4. Add 50 µL of 0.1 mg/mL methaqualone to each tube
5. Add 50 µL of base test mix to the positive control
6. Add approximately 10 mL of n-butyl chloride to each tube, cap tightly and place tubes on rotator for approximately 15 minutes or until extracted
7. Remove tubes from rotator, place in centrifuge for approximately 5 minutes or until separated
8. Pipette the top layer (n-butyl chloride) from each tube into a clean, similarly labeled 15 mL tube
9. Add approximately 5 mL of 1N hydrochloric acid to each tube, cap tightly and repeat the above rotation and centrifugation steps
10. Carefully aspirate and discard the top layer, retaining the lower HCl layer
11. Add 1 mL concentrated ammonium hydroxide to each tube, followed by 100 µL of chloroform, cap and repeat the rotation and centrifugation steps
12. Carefully transfer the bottom (chloroform) layer from each extract to a properly labeled autosampler vial and crimp on the septum cap
13. Place the vials into the autosampler tray and set up a sequence in the data system ensuring that a blank is injected before each sample or control run to detect possible carryover from one specimen to the next
14. Run the sequence, then compare relative retention times and mass spectra of peaks within the chromatograms to known relative retention times and mass spectra, if known

## Quality Assurance

Any chromatographic problems should be investigated and appropriate action taken.

SIM ions may be added to the method so long as the base test mix still performs adequately.

Efforts should be made to keep one milliliter of specimen in reserve, if possible, for further testing.

## Precautions and Notes

Morphine, hydromorphone, and cannabinoids will not be detected with this screening procedure. Screening and quantitation for morphine and hydromorphone can be done with the Opiates procedure. Analysis for cannabinoids can be done with the urine cannabinoids confirmation.

Care should be taken that only fresh concentrated ammonium hydroxide is used to ensure consistent extraction efficiencies.

The specimens may also be extracted by inversion in lieu of using the tube extractor. The samples must be extracted in a manner equivalent to rotation.

### **Preparation of Materials**

#### *1N Hydrochloric acid:*

To a 1 L volumetric, add approximately 500 mL of deionized water. Slowly add 83.0 mL concentrated hydrochloric acid and vortex gently. Dilute to the mark with deionized water and mix well.

#### *Methaqualone working solution (0.10 mg/mL):*

Dilute 1.0 mL stock to 10.0 mL with A.C.S. grade methanol

#### *Base test mix:*

Aliquot 1 mL each of 1 mg/mL certified solutions of amphetamine, methamphetamine, diphenhydramine, amitriptyline, nortriptyline, oxycodone, and alprazolam into a class A 10 mL volumetric flask. Make up to 10 mL with methanol.

## 6.7.4: Acid Screen

### Principle

This method is designed to detect acidic drugs by gas chromatography or gas chromatography-mass spectrometry. The drugs are extracted from their biological matrix by liquid-liquid extraction and identified by their mass spectrum and relative retention time (if known).

### Specimens

This method is applicable to urine, blood, serum, bile, tissue homogenates, and gastric contents. A 5 mL or 5 g sample is generally used unless circumstances warrant the use of a different sample size (e.g. very high or very low suspected drug levels).

### Chemicals and Reagents

Methanol (ACS grade)  
Potassium phosphate monobasic (A.C.S. certified)  
Ethyl ether (chromatographic grade)  
Toluene (chromatographic grade)  
Water (reverse osmosis or Millipore)  
Absolute ethanol  
Hexane (chromatographic grade)  
Compressed inert gas (generally nitrogen or helium)  
Ether/toluene extraction solvent  
80% ethanol extraction solvent

### Controls

Barbital stock solution (20 mg/mL)  
Barbital working solution (0.2 mg/mL)  
Acid test mix: 0.2 mg/mL butalbital, carisoprodol, and phenytoin

### Equipment

15 mL screw cap centrifuge tubes  
Pipets and pipettors  
Tube rotator  
Heating Block  
Centrifuge  
5 mL conical centrifuge tubes  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper  
Nitrogen distribution device  
Vortex mixer

### Instrumentation

Gas chromatograph:	Agilent 6890 or equiv
Mass spectrometer:	Agilent 5973 or equiv
Autosampler:	Agilent 7683 or equiv

Column type:	ZB-5 or equiv
Length (m):	15
ID (mm):	0.25
Film thickness (µm):	0.25

### Instrument conditions

Inlet	
Mode:	Pulsed splitless

Column	
Mode:	Constant pressure

Inlet temp (°C):	250
Pressure (psi):	10.0 (variable)
Pulse pressure (psi):	20.0
Pulse time (min):	0.50
Purge flow (mL/min):	20.0
Purge time (min):	2.00
Total flow (mL/min):	25.2
Gas saver:	On
Saver flow (mL/min):	20.0
Saver time (min):	2.00
Gas type:	Helium

Pressure (psi):	10.00
Initial flow (mL/min):	2.4
Avg. velocity (cm/sec):	81
Detector	
Detector:	MSD
Transfer line temp (°C):	280
Quad temp (°C):	150
Source temp (°C):	230
Mass range (amu):	35-550
Threshold:	150
Number of samples:	2
Solvent delay (min):	2.45 (variable)

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	100	0
15	280	10

## Procedure

1. Label the proper number of 15 mL extraction tubes for the samples and controls to be extracted
2. Pipette 5 mL of body fluid, control, blank, or 5 g 1:1 tissue homogenate (w/w in normal saline) into the labeled tubes
3. Add approximately 0.2 g potassium phosphate monobasic to each tube
4. Add 100 µL of the barbitol internal standard solution to each tube
5. Add 100 µL of the acid test mix to the positive control
6. Add approximately 5 mL of ether/toluene extraction solvent to each tube, cap tightly and place tubes on rotator for approximately 15 minutes or until extracted
7. Remove tubes from rotator, place in centrifuge for approximately 5 minutes or until separated
8. Carefully transfer the top layer (ether/toluene) into properly labeled and solvent-rinsed extraction tubes
9. Place tubes in heating block at approximately 70°C. and evaporate to dryness with nitrogen
10. Add approximately 1 mL of hexane to each tube and vortex
11. Add 100 µL of 80% ethanol to each tube and vortex or stopper the tubes and thoroughly mix by repeated inversions
12. Centrifuge for approximately 5 minutes or until separated
13. Carefully transfer the bottom layer of each tube into the insert of a properly labeled autosampler vial and cap the vial
14. Place vials in autosampler tray and set up a sequence ensuring that a blank is injected before each sample or control run to detect possible carryover from one specimen to the next
15. Run the sequence, then compare relative retention times and mass spectra of peaks within the chromatograms to known relative retention times and mass spectra, if known

## Quality Assurance

Any chromatographic problems should be investigated and appropriate action taken.

Efforts should be made to keep one milliliter of specimen in reserve, if possible, for further testing.

## Precautions and Notes

The specimens may also be extracted by inversion in lieu of using the tube extractor. The samples must be extracted in a manner equivalent to rotation.

## **Preparation of Materials**

### *Barbital stock solution (20 mg/mL):*

Add 200 mg barbital to a Class A 10 mL volumetric flask and make up to 10 mL with A.C.S. grade methanol

### *Barbital working solution (0.2 mg/mL):*

1:100 dilution of 20 mg/mL stock solution in deionized water

### *Ether/toluene extraction solvent:*

1:1 mixture of diethyl ether and toluene

### *80% ethanol extraction solvent:*

80 mL ethanol mixed with 20 mL deionized water

### *Acid test mix:*

Aliquot 2 mL each of 1 mg/mL certified solutions of butalbital, carisoprodol, and phenytoin into a class A 10 mL volumetric flask. Make up to 10 mL with methanol.



### 6.7.5: Quantitation

Many cases where drugs are detected will require quantitation. Quantitations are normally run on blood, gastric contents, or tissue homogenates. Quantitations are not performed on urine specimens because of the difficulty in determining the relevance of the results of that quantitation. The needs of the case determine which samples are quantitated.

Quantitation is generally carried out on a gas chromatograph (GC) or gas chromatograph-mass spectrometer (GC-MS). A calibration curve is created from at least three standards of varying concentration that encompass the concentration range of interest. This range is normally from the low therapeutic range to the lethal range. These standards are prepared in blank blood using certified reference materials. If no certified reference materials are available other sources are acceptable, but the identity of the analyte must then be determined independently and a disclaimer should appear on the report of laboratory analysis stating that a secondary standard was used as the basis of the quantitation of the analyte in question. Use of calibrated analytical balances, pipettors, and class A volumetric glassware, as appropriate, is required for standard preparation. The calibration curve samples must be extracted and analyzed in the same manner as case samples.

Two aliquots of each case specimen should be run if sample amount permits. The quantitative results of any two aliquots of the same specimen should not deviate more than 20% from their mean.

At least one control should be extracted and analyzed in the same manner. This control must be prepared from a different source than the calibration curve, and a record of the source (e.g. lot number, date of acquisition/preparation, standard number) must be kept. The measured value may differ by up to 20% from the known value or fall within the range supplied by the manufacturer.

If the measured value of a control falls outside of the acceptable range, further investigation to determine the source of the discrepancy and appropriate action to correct it is warranted. This normally requires the extraction and analysis of another control or a new control from a third source, if possible. If it is demonstrated that the curve is in error then a new calibration curve must be constructed. If it is demonstrated that the error is confined to the original control sample(s) then the cases may be quantitated against the curve. If a problem in the extraction is demonstrated, then re-extraction of the samples is necessary. The concentration of all control samples must be contained within the calibration curve, or within 20% of the endpoints, or within 30% of the low point of the curve if the control is the same concentration as that low point.

## 6.7.6: Urine Cannabinoid Confirmation

### Principle

This method is designed to detect the presence of ( $\pm$ )-11-nor-9-Carboxy- $\Delta^9$ -THC by selected ion monitoring (SIM) gas chromatography-mass spectrometry. The ( $\pm$ )-11-nor-9-Carboxy- $\Delta^9$ -THC is extracted from its biological matrix by liquid-liquid extraction, derivatized, and detected by monitoring of the derivative ions.

### Specimens

This method is applicable to urine specimens. A 2 mL sample is generally used.

### Chemicals and Reagents

60% KOH

Concentrated acetic acid

Extraction solvent (9:1 hexane:ethyl acetate)

MSTFA

Anhydrous ethyl acetate

### Controls

( $\pm$ )-11-nor-9-Carboxy- $\Delta^9$ -THC certified standard

( $\pm$ )-11-nor-9-Carboxy- $\Delta^9$ -THC- $D_3$  certified standard

### Equipment

15 mL screw cap extraction tubes

Pipets and pipettors

Tube rotator

Centrifuge

Autosampler vials with inserts and crimp-on caps with rubber septa

Crimper

### Instrumentation

Gas chromatograph:	Agilent 6890 or equiv
Mass spectrometer:	Agilent 5973 or equiv
Autosampler:	Agilent 7673 or equiv

Column type:	DB-5MS or equiv
Length (m):	30
ID (mm):	0.20
Film thickness ( $\mu$ m):	0.33

### Instrument conditions

Inlet	
Mode:	Splitless
Inlet temp ( $^{\circ}$ C):	250
Column head pressure (kPa):	125
Gas type:	Helium

Detector	
Detector:	MSD
Transfer line temp ( $^{\circ}$ C):	300
Resolution:	Low
Dwell per ion (ms):	100
Solvent delay (min):	8.9 (variable)

Temperature Ramp		
Rate ( $^{\circ}$ C/min)	Temperature ( $^{\circ}$ C)	Time (min)
	180	0
30	280	10
30	300	2

Ions monitored:	371, 473, 488, 374, 476, 491
-----------------	------------------------------

### Procedure

1. Pipet 2 mL of each specimen or blood bank blood into a 15 mL screw cap extraction tube
2. Add 0.5 µg (±)-11-nor-9-Carboxy-Δ<sup>9</sup>-THC-D<sub>3</sub> to each vial
3. Add 0.2 µg and 0.5 µg (±)-11-nor-9-Carboxy-Δ<sup>9</sup>-THC to the appropriate positive control samples
4. Add 100 µL of 60% KOH
5. Vortex and let stand for approximately 20 minutes
6. Add 500 µL of concentrated acetic acid and vortex
7. Add approximately 2 mL of the extraction solvent
8. Cap and extract on rotator for approximately 10 minutes or until extracted
9. Centrifuge until separated
10. Remove top solvent layer to a 15 mL screw cap extraction tube
11. Evaporate to dryness under nitrogen
12. Add 40 µL of MSTFA
13. Cap and heat at approximately 70°C for approximately 20 minutes
14. Add 100 µL of anhydrous ethyl acetate, vortex, and transfer to an autosampler vial
15. Place vials in autosampler tray and set up a sequence ensuring that a blank is injected before each sample or control run to detect possible carryover from one specimen to the next.
16. Run the sequence using a SIM method that monitors m/z 371, 374, 473, 476, 488, and 491.

### Precautions and Notes

Methanol must not be used to rinse the autoinjector syringe because it hydrolyzes the derivatives formed in this procedure. Anhydrous ethyl acetate should be used instead.

For cannabinoids to be reported as present, the immunoassay urine screen and urine cannabinoids confirmation must both show the presence of cannabinoids.

The specimens may also be extracted by inversion in lieu of using the tube extractor. The samples must be extracted in a manner equivalent to rotation.

This method is qualitative, so quantitative results must not be reported. It is only used to confirm the presence or absence of (±)-11-nor-9-Carboxy-Δ<sup>9</sup>-THC in a specimen.

### Preparation of Materials

#### *60% KOH*

Add 60 g potassium hydroxide slowly to approximately 75 mL deionized water with stirring, being careful not to generate too much heat. When the solid has dissolved, make up to 100 mL of solution with deionized water.

#### *Extraction solvent (9:1 hexane:ethyl acetate)*

Combine 9 parts hexane and 1 part ethyl acetate and mix well.

### 6.7.7: Morphine and 6-Monoacetylmorphine with MBTFA (Liquid Extraction)

#### Principle

This method is designed to detect the presence and quantitative amount of morphine and 6-monoacetylmorphine, which are extracted from their biological matrix by liquid-liquid extraction, derivatized, and detected by SIM monitoring of the ions of the derivatives.

#### Specimens

This method is applicable to blood, urine, tissue homogenates, and other biological fluids.

#### Chemicals and Reagents

Concentrated acetic acid  
Hexane (A.C.S. grade)  
Concentrated hydrochloric acid (A.C.S. grade)  
Absolute methanol  
Sodium carbonate (A.C.S. grade)  
Sodium hydroxide (A.C.S. grade)  
Toluene (A.C.S. grade)  
Isoamyl alcohol (A.C.S. grade)  
Ethyl acetate (A.C.S. grade)  
Deionized water  
pH 9.1 carbonate buffer  
Extraction solvent (78:20:2 toluene:hexane:isoamyl alcohol)  
8 N sodium hydroxide  
N-methyl-bis(trifluoroacetamide) (MBTFA)

#### Controls

Certified standards for morphine, 6-monoacetylmorphine, and nalorphine

#### Equipment

15 mL screw cap extraction tubes with caps  
5 mL conical centrifuge tubes with caps  
Rotator  
Vortex mixer  
Heating block  
Evaporation manifold  
Class A pipets and volumetric flasks  
Analytical balance  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper

#### Instrumentation

Gas chromatograph:	Agilent 5890 or equiv
Mass spectrometer:	Agilent 5971 or equiv
Autosampler:	Agilent 7673 or equiv

Column type:	DB-5MS or equiv
Length (m):	30
ID (mm):	0.20
Film thickness (µm):	0.33

#### Instrument conditions

Inlet
-------

Detector
----------

Mode:	Splitless
Inlet temp (°C):	250
Column head pressure (kPa):	125
Gas type:	Helium

Detector:	MSD
Transfer line temp (°C):	280
Resolution:	High
Dwell per ion (ms):	100
Solvent delay (min):	12 (variable)

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	100	2
20	280	5
30	300	4

Ions Monitored	
Morphine:	364, 477
6-Monoacetylmorphine:	364, 423
Nalorphine:	390, 503

## Procedure

1. Label the proper number of 15mL extraction tubes
2. Add 4 mL of blank blood to the tubes for standards and controls
3. Add the appropriate amount of each analyte to the tubes for each calibration curve point
4. Add 4 mL of specimen into the appropriate tubes
5. Add 100 µL of 10µg/mL nalorphine to each tube as an internal standard
6. Add 4 mL of pH 9.1 sodium carbonate buffer to each tube
7. Add 5 mL of extraction solvent to each tube
8. Cap and extract on rotator for approximately 10 minutes or until extracted
9. Remove tubes and centrifuge until separated
10. Transfer the top organic layer of each tube to a properly labeled 15 mL screw cap extraction tube
11. Evaporate to dryness under inert gas with the evaporation manifold at approximately 60-70°C
12. Add 40 µL MBTFA to each tube, cap, vortex, and heat at approximately 60-70°C for 20 minutes
13. Add 150 µL of anhydrous ethyl acetate to each tube, vortex, and transfer to an appropriately labeled autosampler vial equipped with an insert
14. Place vials in autosampler tray and set up a sequence ensuring that a blank is injected before each sample or control run to detect possible carryover from one specimen to the next
15. Run the sequence using a SIM method that monitors m/z 364, 390, 423, 477, and 503 throughout the run.

## Quality Assurance

The calibration curve should extend from 0.025 µg/mL to 2 µg/mL for each analyte. The calibration curve must have a correlation coefficient of at least 0.990 and the measured value of no curve point may vary more than 20% from the known value of that curve point, with the exception of the lowest point on the curve, which may vary up to 30% from its known value.

At least one control should be extracted with the samples and analyzed in the same manner. This control must be prepared from a different source than the calibration curve. The measured value may differ by up to 20% from the known value.

If morphine is determined to be present, the ions for 6-monoacetylmorphine must be scanned to determine whether it is also present. Standards for 6-monoacetylmorphine must also be run

## Precautions and Notes

Methanol must not be used to rinse the autoinjector syringe because it hydrolyzes the derivatives formed in this procedure. Anhydrous ethyl acetate should be used instead.

Reinjection of samples must occur within 12 hours of the original injection due to breakdown of the derivatives formed in this procedure.

Deuterated internal standards may be used in lieu of nalorphine if their ions are added to the SIM ion list.

The following ions should be used to identify any drugs present:

Drug	Quantitation ion	Qualifier ion
Morphine	364	477
6-Monoacetylmorphine	364	423
Nalorphine	390	503

To confirm a drug as present, the ratio of the intensities of the quantitation ion and the qualifier ion must not vary more than 20% relative to the ratio of these two ions in the control samples. If co-elution is suspected, the chromatographic parameters may be changed in order to remove the interference.

The specimens may also be extracted by inversion in lieu of using the tube extractor. The samples must be extracted in a manner equivalent to rotation.

## Preparation of Materials

### *pH 9.1 carbonate buffer*

Add 10.6 g  $\text{Na}_2\text{CO}_3$  to a class A 100 mL volumetric flask and dilute to the mark with deionized water. Adjust to pH 9.1 with acetic acid. Stable for 6 months at room temperature.

### *Extraction solvent (78:20:2 toluene:hexane:isoamyl alcohol)*

Mix 78 mL toluene, 20 mL hexane, and 2 mL isoamyl alcohol. Stable for one month at room temperature.

### *8N sodium hydroxide*

Add 32 grams NaOH to a class A 100 mL volumetric flask and slowly add sufficient deionized water to make up to the line, ensuring the solution does not become too hot.

### 6.7.8: Hydromorphone with MBTFA (Liquid Extraction)

#### Principle

This method is designed to detect the presence and quantitative amount of hydromorphone, which is extracted from its biological matrix by liquid-liquid extraction, derivatized, and detected by SIM monitoring of the ions of its derivative.

#### Specimens

This method is applicable to blood, urine, tissue homogenates, and other biological fluids.

#### Chemicals and Reagents

Concentrated acetic acid  
Hexane (A.C.S. grade)  
Concentrated hydrochloric acid (A.C.S. grade)  
Absolute methanol  
Sodium carbonate (A.C.S. grade)  
Sodium hydroxide (A.C.S. grade)  
Toluene (A.C.S. grade)  
Isoamyl alcohol (A.C.S. grade)  
Ethyl acetate (A.C.S. grade)  
Deionized water  
pH 9.9 carbonate buffer  
Extraction solvent (78:20:2 toluene:hexane:isoamyl alcohol)  
8 N sodium hydroxide  
N-methyl-bis(trifluoroacetamide) (MBTFA)

#### Controls

Certified standards for morphine, hydromorphone, codeine, 6-monoacetylmorphine, and nalorphine

#### Equipment

15 mL screw cap extraction tubes with caps  
5 mL conical centrifuge tubes with caps  
Rotator  
Vortex mixer  
Heating block  
Evaporation manifold  
Class A pipets and volumetric flasks  
Analytical balance  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper

#### Instrumentation

Gas chromatograph:	Agilent 5890 or equiv
Mass spectrometer:	Agilent 5971 or equiv
Autosampler:	Agilent 7673 or equiv

Column type:	DB-5MS or equiv
Length (m):	30
ID (mm):	0.20
Film thickness (µm):	0.33

#### Instrument conditions

Inlet	
Mode:	Splitless

Detector	
Detector:	MSD

Inlet temp (°C):	250
Column head pressure (kPa):	125
Gas type:	Helium

Transfer line temp (°C):	280
Resolution:	High
Dwell per ion (ms):	100
Solvent delay (min):	12 (variable)

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	100	2
20	280	5
30	300	4

Ions Monitored	
Hydromorphone:	325, 381
Nalorphine:	390, 503

## Procedure

1. Label the proper number of 15mL extraction tubes
2. Add 4 mL of blank blood to the tubes for standards and controls
3. Add the appropriate amount of each analyte to the tubes for each calibration curve point
4. Add 4 mL of specimen into the appropriate tubes
5. Add 100 µL of 10µg/mL nalorphine to each tube as an internal standard
6. Add 4 mL of pH 9.9 sodium carbonate buffer to each tube
7. Add 5 mL of extraction solvent to each tube
8. Cap and extract on rotator for approximately 10 minutes or until extracted
9. Remove tubes and centrifuge until separated
10. Transfer the top organic layer of each tube to a properly labeled 15 mL screw cap extraction tube
11. Evaporate to dryness under inert gas with the evaporation manifold at approximately 60-70°C
12. Add 40 µL MBTFA to each tube, cap, vortex, and heat at approximately 60-70°C for 20 minutes
13. Add 150 µL of anhydrous ethyl acetate to each tube, vortex, and transfer to an appropriately labeled autosampler vial equipped with an insert
14. Place vials in autosampler tray and set up a sequence ensuring that a blank is injected before each sample or control run to detect possible carryover from one specimen to the next
  - o Run the sequence using a SIM method that monitors m/z 325, 364, 381, 390, 477, and 503 throughout the run.

## Quality Assurance

The calibration curve should extend from 0.025 µg/mL to 2 µg/mL for each analyte. The calibration curve must have a correlation coefficient of at least 0.990 and the measured value of no curve point may vary more than 20% from the known value of that curve point, with the exception of the lowest point on the curve, which may vary up to 30% from its known value.

At least one control should be extracted with the samples and analyzed in the same manner. This control must be prepared from a different source than the calibration curve. The measured value may differ by up to 20% from the known value.

## Precautions and Notes

Hydromorphone may also be extracted using the same method as for morphine and 6-monoacetylmorphine. All samples and controls must be extracted using the same extraction method.

Methanol must not be used to rinse the autoinjector syringe because it hydrolyzes the derivatives formed in this procedure. Anhydrous ethyl acetate should be used instead.



Reinjection of samples must occur within 12 hours of the original injection due to breakdown of the derivatives formed in this procedure.

Samples must not be derivatized at a higher temperature or a longer time than listed. Doing so may cause the formation of a di-TMS derivative identical to that of derivatized morphine instead of the mono-TMS hydromorphone derivative.

Deuterated internal standards may be used in lieu of nalorphine if their ions are added to the SIM ion list.

The following ions should be used to identify any drugs present:

Drug	Quantitation ion	Qualifier ion
Hydromorphone	325	381
Nalorphine	390	503

To confirm a drug as present, the ratio of the intensities of the quantitation ion and the qualifier ion must not vary more than 20% relative to the ratio of these two ions in the control samples. If co-elution is suspected, the chromatographic parameters may be changed in order to remove the interference.

The specimens may also be extracted by inversion in lieu of using the tube extractor. The samples must be extracted in a manner equivalent to rotation.

### **Preparation of Materials**

#### *pH 9.9 carbonate buffer*

Add 10.6 g Na<sub>2</sub>CO<sub>3</sub> to a class A 100 mL volumetric flask and dilute to the mark with deionized water. Adjust to pH 9.9 with acetic acid. Stable for 6 months at room temperature.

#### *Extraction solvent (78:20:2 toluene:hexane:isoamyl alcohol)*

Mix 78 mL toluene, 20 mL hexane, and 2 mL isoamyl alcohol. Stable for one month at room temperature.

#### *8N sodium hydroxide*

Add 32 grams NaOH to a class A 100 mL volumetric flask and slowly add sufficient deionized water to make up to the line, ensuring the solution does not become too hot.

## 6.7.9: Reinsch Screen

### Principle

This method is designed to screen for arsenic and other heavy metals in blood, urine, or tissue specimens. The specimens are heated in strong acid, and any arsenic will collect on a copper wire inserted in the sample. A dull deposit on the wire indicates a positive result.

### Specimens

This method is applicable to blood, urine, vitreous, bile, gastric contents, or tissue homogenate. Specimen amount varies according to specimen availability, but 15 mL of blood, urine, vitreous, or bile, or 5 g of gastric contents or tissue homogenate is generally used.

### Chemicals and Reagents

Concentrated nitric acid

Concentrated hydrochloric acid

Deionized water

Coiled copper wires (copper wire wrapped 10 times around a glass rod or other similarly-sized cylinder)

Blood bank blood

### Controls

Arsenic working standard (100 µg/mL)

### Equipment

Hot plate

Water bath

50 mL Erlenmeyer flasks

### Procedure

1. Pipet 15 mL of blank blood into two 50 mL Erlenmeyer flasks. Label as 'Standard' and 'Blank'.
2. Add specimen (15 mL blood or 5 g tissue) to a 50 mL Erlenmeyer flask labeled with the case number
3. Add 200 µL of the 100 µg/mL working standard to the flask labeled 'Standard'
4. Add deionized water to each flask until the volume of all samples is 20 mL
5. Add 3 mL concentrated hydrochloric acid to each flask
6. Clean one copper wire for every flask by immersing the wire in concentrated nitric acid for 5 seconds
7. Rinse each wire well with deionized water and place one wire in each flask
8. Place the flasks in a heated water bath and heat gently for approximately 2 hours
9. Remove the wires and gently rinse them thoroughly with deionized water
10. Examine the wires carefully. A dull deposit on the wire indicates the presence of a heavy metal.

### Quality Assurance

Outside testing is required to identify any heavy metal present.

### Precautions and Notes

Sufficient sample to send for outside testing should be set aside if identification of any heavy metal present is required.

This procedure is taken with modification from *Clarke's Isolation and Identification of Drugs*, A. C. Moffat, J. V. Jackson, M. S. Moss, B. Widdop, The Pharmaceutical Press, Second Edition, 1986

### **Preparation of Materials**

#### *2N Hydrochloric acid*

In a 250 mL volumetric flask, place approximately 100 mL of deionized water. Slowly add 42 mL of concentrated hydrochloric acid and gently vortex. Dilute to the mark with deionized water and mix well.

#### *Arsenic stock standard (1 mg/mL)*

In a 100 mL volumetric flask, place 0.132 grams of  $\text{As}_2\text{O}_3$  (or an equimolar amount of another arsenic<sup>3+</sup> salt). Add 16.6 mL of concentrated hydrochloric acid. Dilute to the mark with deionized water and mix well.

#### *Arsenic working standard (100 µg/mL)*

In a 100 mL volumetric flask, place 10 mL of the 1 mg/mL arsenic stock standard. Dilute to the mark with 2N hydrochloric acid and mix well.

## 6.7.10: Cyanide screen and quantitation

### Principle

This method is designed to detect and quantitate the cyanide ion in blood or other biological samples using spectrophotometry.

### Specimens

This method is applicable to blood, urine, vitreous, bile, gastric contents, or tissue homogenate.

Specimen amount varies according to specimen availability, but 1 mL of blood, urine, vitreous, or bile or 1 g of gastric contents or a 1:4 tissue homogenate is generally used.

### Chemicals and Reagents

Deionized water

0.1 N Sodium hydroxide

10% Sulfuric acid

0.25% Chloramine T

Phosphate solution

Barbituric acid reagent (make fresh daily)

Sodium cyanide or potassium cyanide

### Controls

0.50 mg/mL cyanide stock

5.0 µg/mL cyanide control

### Equipment

UV-Visible spectrophotometer

Conway diffusion cells

### Procedure

1. Prepare a Conway diffusion cell for each standards, blank, or specimen to be tested.
2. To the center ring, add 0.5 mL of 0.1 N sodium hydroxide with a micropipettor.
3. To the second ring, add 0.5 mL of 10% sulfuric acid with a micropipettor
4. To the second ring, add 1 mL of each standard or specimen. And immediately seal the dish.
  - a. For the 0.5 µg/mL standard, add 100 µL of the 5.0 µg/mL cyanide control and 900 µL deionized water
  - b. For the 1.0 µg/mL standard, add 200 µL of the 5.0 µg/mL cyanide control and 800 µL deionized water
  - c. For the 2.0 µg/mL standard, add 400 µL of the 5.0 µg/mL cyanide control and 600 µL deionized water
  - d. For the 5.0 µg/mL standard, add 1000 µL of the 5.0 µg/mL cyanide control
  - e. For the blank, add 1000 µL of blank blood
5. Allow the samples to sit for approximately two hours.
6. Transfer 200 µL of the solution in the center ring to a clean, labeled 15 mL extraction tube.
7. Add 1 mL of phosphate solution to each tube.
8. Add 500 µL of Chloramine T solution to each tube.
9. Vortex each tube and allow to sit for approximately three minutes.
10. Add 1.5 mL of barbituric acid reagent to each sample and vortex.
11. Allow the samples to sit for approximately ten minutes.
12. Read the absorbance of each solution at 586 nm.

13. Construct a quantitation curve using the standards and quantitate each case specimen using this curve.

### Precautions and Notes

Cyanide salts and solutions are extremely toxic and great care should be taken in their handling.

The cyanide solutions should be disposed of when no longer needed by placing the cyanide solution in a beaker in an ice bath in a hood, followed by the slow addition of a 5.25% solution (approximately) of sodium hypochlorite. This converts the cyanide to cyanate ( $\text{NaCN} + \text{NaOCl} \rightarrow \text{NaOCN} + \text{NaCl}$ ). Care should be taken that this solution does not get too hot. When a 50% excess of hypochlorite has been added and heat is no longer being evolved, the mixture is allowed to stand for several hours and may be washed down the drain with excess water.

### Preparation of Materials

#### *0.50 N Sodium hydroxide*

Add approximately 50 mL of deionized water to a 100 mL class A volumetric flask. Add 0.40 g sodium hydroxide, making sure that the solution does not get too hot. Dilute to the mark with deionized water and mix well.

#### *10% Sulfuric acid*

Add approximately 10 mL of deionized water to a 25 mL class A volumetric flask. Slowly add 2.5 mL of concentrated sulfuric acid, taking care that the solution does not get too hot. Dilute to the mark with deionized water and mix well.

#### *0.25% Chloramine T*

Add approximately 10 mL of deionized water to a 25 mL class A volumetric flask. Add 0.0625 g of Chloramine T. Dilute to the mark with deionized water and mix well.

#### *Phosphate solution*

Add approximately 10 mL of deionized water to a 25 mL class A volumetric flask. Add 3.45 g of sodium phosphate monobasic. Dilute to the mark with deionized water and mix well.

#### *Barbituric acid reagent (make fresh daily)*

Add 7.5 mL of pyridine to a 25 mL class A volumetric flask. Add 1.5 g barbituric acid. Slowly add 1.5 mL of concentrated hydrochloric acid, taking care that the solution does not get too hot. Dilute to the mark with deionized water and mix well.

#### *0.50 mg/mL cyanide stock*

In a 25 mL class A volumetric flask, place approximately 10 mL of deionized water. Add 2 mL of 0.1 N sodium hydroxide. Add 0.0313 g of potassium cyanide or 0.0235 g of sodium cyanide. Dilute to the mark with deionized water and mix well.

#### *5.0 µg/mL cyanide control*

In a 25 mL class A volumetric flask, add 250 µL of the 0.50 mg/mL cyanide stock solution. Dilute to the mark with deionized water and mix well.

### **6.7.11: Carbon Monoxide**

The presence of carbon monoxide should be confirmed by two methods if the case history is inconsistent with the results of the assay and if specimen size allows. In each case a negative control consisting of blood bank blood must be analyzed. If the negative control shows the presence of carboxyhemoglobin (COHb) (above 5% saturation) further investigation and appropriate action is warranted. Only samples containing hemoglobin are appropriate to analyze using these methods.

#### **6.7.11.1: Carbon Monoxide by CO-Oximeter**

##### **Principle**

This method is designed to measure carboxyhemoglobin (COHb) levels in blood. The specimens are sampled by a CO-Oximeter and the results are obtained by multi-wavelength spectrophotometry.

##### **Specimens**

This method is applicable to blood. The sample size taken by the CO-Oximeter is approximately 65 µL

##### **Chemicals and Reagents**

Zeroing solution (obtained from IL Instrumentation Laboratory)

Cleaning agent (obtained from IL Instrumentation Laboratory)

Diluent (obtained from IL Instrumentation Laboratory)

##### **Controls**

Positive controls are obtained from IL Instrumentation Laboratory

Blank blood

##### **Equipment**

IL 682 CO-Oximeter

##### **Procedure**

1. Analyze samples and controls according to procedures set forth in manual for the IL 682 CO-Oximeter
2. Blood samples may be drawn into a syringe through a 26-gauge (or smaller) needle before analysis to prevent clogging the tubing or cell in the CO-Oximeter
3. Samples that produce high methemoglobin readings can be treated with small amounts of sodium dithionite and retested. The blood and dithionite should be thoroughly mixed and allowed to stand for two minutes to ensure full reaction.
4. Record sample and control results in appropriate folders

##### **Quality Assurance**

The IL 682 CO-Oximeter can detect problem conditions such as high turbidity or insufficient hemoglobin. Results obtained under these conditions are suspect and may be discarded.

A positive control obtained from IL Instrumentation Laboratory and a negative control of blank blood are run to ensure that the assay responds appropriately to the presence of carbon monoxide.

Two samples should be run and the results should agree to within 10% of one another. The average of the two results is reported.

The thermal paper printouts generated by the IL 682 CO-Oximeter will be photocopied for retention in the case file. The original printouts will be discarded.

The CO-Oximeter may additionally be cleaned manually between samples with a bleach solution, followed by water, at the discretion of the analyst.

The matrix blank cannot contain more than 5% carboxyhemoglobin saturation. The positive control must be within the range reported by the manufacturer. If either control is out of range, it may be rerun.

Results above or below the range of controls must be reported as “less than” the mean value for the lowest control rounded to the nearest integer, or “greater than” the mean value for the highest control rounded to the nearest integer. In the intermediate range, results are reported as the average saturation value rounded to the nearest integer.

### **Precautions and Notes**

Only samples containing hemoglobin are appropriate to analyze using this method.

### **Preparation of Materials**

No materials are prepared in this method.

### 6.7.11.2: Carbon Monoxide by Diffusion Cell

#### Principle

This method is designed to detect the presence of carboxyhemoglobin (COHb) in blood. The results are determined by the presence or absence of a metallic film in the center well of a Conway diffusion cell.

#### Specimens

This method is applicable to blood. The sample size required is 0.5 mL.

#### Chemicals and Reagents

0.005N Palladium chloride

0.1N Hydrochloric acid

3.6N Sulfuric acid

Light grease

#### Controls

Positive controls are obtained from IL Instrumentation Laboratory.

Blank blood

#### Equipment

Conway diffusion cell with cover

Pipets

Vaseline or other light grease

#### Procedure

1. Pipet 3 mL of 0.005N palladium chloride in 0.1N hydrochloric acid into the center well of each Conway diffusion cell.
2. Pipet 1 mL of 3.6N sulfuric acid into the outer well of each cell.
3. Lightly line the seal of each cell with Vaseline or similar light grease.
4. Without mixing with the acid, add 0.5 mL of the sample or control to be tested to each outer cell, put its cover glass in place and carefully mix the contents of the outer cell. Allow the cells to stand for at least two hours.
5. Appearance of a metallic film on the surface of the inner cell liquid indicates that carbon monoxide was released from the blood. The minimum detection limit of this procedure is considered to be 15% saturation.

#### Quality Assurance

A positive control obtained from IL Instrumentation Laboratory and a negative control of blood bank blood are run to ensure that the assay responds appropriately to the presence of carbon monoxide. The positive control must be clearly more positive than the negative control. The negative control must not have any metallic film.

#### Precautions and Notes

Only samples containing hemoglobin are appropriate to analyze using this method. This test is a screen only and does not give quantitative results.

#### Preparation of Materials

*0.1N Hydrochloric acid*



In a 100 mL volumetric flask add approximately 50 mL of deionized water. Slowly add 8.3 mL of concentrated hydrochloric acid and vortex gently. Dilute up to the mark with deionized water and mix well.

*0.005N Palladium chloride*

Dissolve 0.44 g palladium chloride in 500 mL 0.1N HCl and allow to stand overnight. Dilute to 1 L with 0.1N HCl.

*3.6N Sulfuric acid*

Add 10.0 mL concentrated sulfuric acid to a class A 100 mL volumetric flask and make up to the mark with deionized water.

COPY

### 6.7.11.3: Carbon Monoxide by UV-Vis Spectrometer

#### Principle

This method is designed to detect the presence of carboxyhemoglobin (COHb) in blood. The results are determined by multi-wavelength spectrophotometry using a UV-Visible spectrometer.

#### Specimens

This method is applicable to blood. The sample size required is 0.02 mL.

#### Chemicals and Reagents

Sodium hydrosulfite  
0.1% Sodium carbonate  
5N Sodium hydroxide  
Water  
Blank blood

#### Controls

Positive controls are obtained from IL Instrumentation Laboratory.  
Blank blood

#### Equipment

UV-Visible spectrometer  
Matched cuvettes  
Micropipettes and tips

#### Procedure

1. Turn on the UV-Visible spectrometer and let the lamps warm up before analysis.
2. Add approximately 2 milligrams of solid sodium hydrosulfite to a cuvette containing approximately 2.5 milliliters of 0.1% sodium carbonate.
3. Add 10 microliters of blood and mix.
4. Add 200 microliters of 5N sodium hydroxide to the cuvette and mix.
5. Read the absorbances at 532 and 558 nanometers, with water used as the blank.
6. Calculate the carboxyhemoglobin saturation as:  $67 * (2.44 - A_{558} / A_{532})$
7. Turn off the UV-Visible spectrometer lamps

#### Quality Assurance

A positive control obtained from IL Instrumentation Laboratory and a negative control of blood bank blood are run to ensure that the assay responds appropriately to the presence of carbon monoxide. The positive control must be within the limits established by the manufacturer for the appropriate lot of controls.

Two samples should be run and the results should agree to within  $\pm 3\%$  from the average. The average of the two results is reported.

The matrix blank cannot contain more than 5% carboxyhemoglobin saturation. The positive control must be within the widest listed acceptable range reported by the manufacturer. If either control is out of range, it may be rerun.

Results above or below the range of controls must be reported as "less than" the lowest value for the lowest control rounded to the nearest integer, or "greater than" the highest value for the highest control rounded to the nearest integer. In the intermediate range, results are reported as the average saturation value rounded to the nearest integer.

### **Precautions and Notes**

Only samples containing hemoglobin are appropriate to analyze using this method.

### **Preparation of Materials**

#### *5N Sodium hydroxide:*

Slowly add 100 g of sodium hydroxide to 500 mL of deionized water, stirring gently. Take care that the solution does not become too hot.

#### *0.1% sodium carbonate*

Add 0.25 g of sodium carbonate to 250 mL of deionized water, mixing well.

### **Literature References**

Journal of Forensic Sciences, Vol. 27, No. 4, Oct. 1982, pp. 928-934

## 6.7.12: Gamma-hydroxybutyrate (GHB) screen and quantitation

### Principle

This method is designed to detect the presence of gamma-hydroxybutyrate by mass spectrometry. The gamma-hydroxybutyrate is extracted from its biological matrix by liquid-liquid extraction, derivatized, and detected by monitoring of the derivative ions. Quantitation is performed by comparison to extracted standards. Confirmation of the presence of gamma-hydroxybutyrate is obtained by acquiring a mass spectrum of the derivative.

### Specimens

This method is applicable to urine specimens, although alternate specimens can be used if necessary. A 200 µL sample is generally used.

### Chemicals and Reagents

Anhydrous ethyl acetate  
MSTFA

### Controls

GHB  
GHB-d<sub>6</sub>

### Equipment

Test tubes  
Pipets and pipettors  
Vortex  
Centrifuge  
Evaporation manifold with inert gas source (generally nitrogen)  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper

### Instrumentation

Gas chromatograph:	Agilent 5890 or equiv
Mass spectrometer:	Agilent 5971 or equiv
Autosampler:	Agilent 7673 or equiv

Column type:	DB-5MS or equiv
Length (m):	30
ID (mm):	0.20
Film thickness (µm):	0.33

### Instrument conditions

Inlet	
Mode:	Splitless
Inlet temp (°C):	250
Column head pressure (kPa):	125
Gas type:	Helium

Detector	
Detector:	MSD
Transfer line temp (°C):	280
Resolution:	High
Dwell per ion (ms):	50
Solvent delay (min):	5 (variable)

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	60	0
20	160	0
10	180	0
30	300	5

Ions monitored:	233, 234, 239, 240
-----------------	--------------------

## Procedure

1. Pipet 200 µL of each specimen or blank urine into a properly labeled test tube for each case, curve point, or control
2. Add 100 µL of 50 µg/mL GHB-d<sub>6</sub> internal standard to each tube
3. Add 500 µL methanol to each tube
4. Vortex and centrifuge for approximately 5 minutes or until separated
5. If solid material is precipitated, transfer the supernatant to a new test tube
6. Evaporate each sample to dryness under nitrogen at approximately 40°C (not more than 50°C)
7. Add 75 µL of anhydrous ethyl acetate to each tube
8. Add an extra 75 µL of anhydrous ethyl acetate to the tube for the highest curve point
9. Add 75 µL of MSTFA to each tube
10. Layer with nitrogen and seal each tube with paraffin film
11. Derivatize at approximately 60°C for 30 minutes
12. Transfer each sample to an autosampler vial with an insert
13. Place vials in autosampler tray and set up a sequence ensuring that a blank is injected before each sample or control run to detect possible carryover from one specimen to the next.
14. Run the sequence using a SIM method that monitors m/z 233, 234, 239, and 240.
15. If the presence of GHB at a urine concentration of above 10 µg/mL is indicated, run that sample in scan mode to obtain a full mass spectrum for confirmation, along with the extracted blank and an extracted standard at approximately the same concentration.

## Quality Assurance

The ratio of the qualifier ion(s) relative to the quantitation ion should not differ more than 20%. If the ion ratios differ more than 20%, the presence can still be confirmed by comparison of the full mass spectra.

The retention time of any analyte should not differ more than 2% from the retention time of its control.

Curve points must be run bracketing the concentration of 10 µg/mL. A suggested curve is:

Curve point (GHB Na salt)	(GHB)	Add to tube
0 µg/mL	0 µg/mL	Blank urine only
2.5 µg/mL	2.044 µg/mL	50 µL of 10 µg/mL GHB sodium salt
5 µg/mL	4.088 µg/mL	100 µL of 10 µg/mL GHB sodium salt
10 µg/mL	8.177 µg/mL	200 µL of 10 µg/mL GHB sodium salt
20 µg/mL	16.35 µg/mL	40 µL of 100 µg/mL GHB sodium salt
40 µg/mL	32.71 µg/mL	80 µL of 100 µg/mL GHB sodium salt

Controls should be run at approximately 10 µg/mL and at approximately 40 µg/mL. A suggested addition is:

Control (GHB Na salt)	(GHB)	Add to tube
10 µg/mL	8.177 µg/mL	40 µL of 50 µg/mL GHB sodium salt
25 µg/mL	20.44 µg/mL	100 µL of 50 µg/mL GHB sodium salt

Any urine specimen in which GHB is present at above 10 µg/mL in the urine must be confirmed by comparing the full mass spectrum with that of an extracted standard, and contrasting with an extracted blank.

### Precautions and Notes

Methanol must not be used to rinse the autoinjector syringe because it hydrolyzes the derivatives formed in this procedure. Anhydrous ethyl acetate should be used instead.

The molecular weight of GHB is 103.0975 amu. The molecular weight of GHB sodium salt is 126.0873 amu. To convert a concentration from GHB sodium salt to GHB, multiply the concentration by 0.8177.

This method is taken with modification from *Application of a Convenient Extraction Procedure to Analyze Gamma-Hydroxybutyric Acid in Fatalities Involving Gamma-Hydroxybutyric Acid, Gamma-Butyrolactone, and 1,4-Butanediol* by W.C. Duer, K.L. Byers, and J.V. Martin (Journal of Analytical Toxicology, Volume 25, October 2001, pp. 576-582).

### Preparation of Materials

#### *50 µg/mL GHB-d<sub>6</sub>*

Add 500 µL of a 1 mg/mL certified standard to a 10 mL class A volumetric flask and make up with methanol.

#### *50 µg/mL GHB sodium salt (81.77 µg/mL GHB) from certified standard*

Add 500 µL of a 1 mg/mL certified standard to a 10 mL class A volumetric flask and make up with methanol.

#### *100 µg/mL GHB sodium salt (81.77 µg/mL GHB) from powder*

Add 0.0250 g GHB sodium salt to a 25mL class A volumetric flask and make up with methanol.

## 6.7.13: Cocaine and Benzoylecgonine by Solid Phase Extraction and Gas Chromatography-Mass Spectrometry

### Principle

This method is designed to detect the presence and amount of cocaine and benzoylecgonine. The analytes are extracted from their biological matrix by liquid-liquid extraction, derivatized, and detected by monitoring of selected ions. Quantitation is performed by comparison to extracted standards.

### Specimens

This method is applicable with all specimen types, provided that the standards and controls are prepared in the appropriate matrix.

### Chemicals and Reagents

Deionized water  
Methanol  
Ammonium hydroxide  
Acetonitrile  
Na<sub>2</sub>HPO<sub>4</sub> (Sodium phosphate, dibasic)  
NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (Sodium phosphate monobasic monohydrate)  
Hydrochloric acid  
Elution solvent  
BSTFA with 1% TMCS

### Controls

Cocaine in acetonitrile  
Benzoylecgonine in acetonitrile  
0.01 µg/mL Cocaine-d<sub>3</sub> in acetonitrile  
0.01 µg/mL Benzoylecgonine-d<sub>3</sub> (BE-d<sub>3</sub>) in acetonitrile

### Equipment

SPE Manifold  
Mixed-mode SPE tube (octyl and benzenesulfonic acid, such as UCT Clean Screen CSDAU206)  
Test tubes  
Pipets and pipettors  
Vortex  
Centrifuge  
Evaporation manifold with inert gas source (generally nitrogen)  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper

### Instrumentation

Gas chromatograph:	Agilent 6890 or equiv
Mass spectrometer:	Agilent 5973 or equiv
Autosampler:	Agilent 7683 or equiv

Column type:	ZB-5 or equiv
Length (m):	15
ID (mm):	0.25
Film thickness (µm):	0.25

### Instrument conditions

Inlet
-------

Column
--------

Mode:	Pulsed splitless
Inlet temp (°C):	250
Pressure (psi):	10.0 (variable)
Pulse pressure (psi):	20.0
Pulse time (min):	0.50
Purge flow (mL/min):	20.0
Purge time (min):	2.00
Total flow (mL/min):	25.2
Gas saver:	On
Saver flow (mL/min):	20.0
Saver time (min):	2.00
Gas type:	Helium

Mode:	Constant pressure
Pressure (psi):	10.00
Initial flow (mL/min):	2.4
Avg. velocity (cm/sec):	81
Detector	
Detector:	MSD
Transfer line temp (°C):	280
Quad temp (°C):	150
Source temp (°C):	230
Mass range (amu):	35-550
Threshold:	150
Number of samples:	2
Solvent delay (min):	2.45 (variable)

Temperature Ramp		
Rate (C°/min)	Temperature (°C)	Time (min)
	100	0
15	280	10

## Procedure

### 1. Sample Preparation

- To one milliliter of specimen, add 50 µL of internal standard and 4 mL of deionized water.
- Mix or vortex and let stand for 5 minutes.
- Centrifuge for 5 minutes and discard pellet (if present).
- Add 2 mL 0.1M phosphate buffer (pH 6.0) and mix or vortex.
- The sample pH should be 6.0±0.5. Adjust if necessary with Na<sub>2</sub>HPO<sub>4</sub> (lowers pH) or NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (raises pH).

### 2. Column Conditioning

- Add 3 mL methanol and aspirate to waste at low pressure (less than 3 in Hg).
- Add 3 mL deionized water and aspirate to waste at low pressure (less than 3 in Hg).
- Add 1 mL 0.1M phosphate buffer (pH 6.0) and aspirate to waste at low pressure (less than 3 in Hg).
- Ensure that the column does not dry after conditioning.

### 3. Load prepared specimen into SPE column and aspirate at 1 to 2 mL/minute.

### 4. Column Washing

- Add 2 mL deionized water and aspirate to waste at low pressure (1-2 mL/min).
- Add 2 mL 0.1M hydrochloric acid and aspirate to waste at low pressure (1-2 mL/min).
- Add 3 mL methanol and aspirate to waste at low pressure (1-2 mL/min).
- Completely dry the column under high pressure (>10 in Hg).

### 5. Elution

- Add 3 mL of elution solvent and collect eluate at low pressure (1-2 mL/min).

### 6. Sample Derivatization

- Dry eluate at less than 40°C.
- Add 50 µL ethyl acetate.
- Add 50 µL BSTFA with 1% TMCS.
- Overlay with dry nitrogen, cap, and mix or vortex.
- Derivatize for 20 minutes at 70°C.
- Remove from heat source and let cool.

### 7. Analysis

- Inject on GC-MS in SIM mode monitoring the ions:



Drug	Quant Ion	Qualifier Ion 1	Qualifier Ion 2
Cocaine	182	303	272
Cocaine-d3	185	306	275
BE	240	361	256
BE-d3	243	364	259

The suggested standard curve consists of the points 0.05, 0.1, 0.5, 1.0, and 1.5 µg/mL of each analyte. Two positive controls must be run with each batch and hit within 20% of the target value. An extracted blank containing internal standard must be run with each batch, and not contain any drug above the limit of quantitation.

### Quality Assurance

The ratio of the qualifier ion(s) relative to the quantitation ion should not differ more than 20%. If the ion ratios differ more than 20%, the presence can still be confirmed by comparison of the full mass spectra.

The retention time of any analyte should not differ more than 2% from the retention time of its control.

The suggested standard curve consists of the points 0.05, 0.1, 0.5, 1.0, and 1.5 µg/mL of each analyte. Two positive controls must be run with each batch and hit within 20% of the target value. An extracted blank containing internal standard must be run with each batch, and not contain any drug above the lower limit of quantitation.

Results obtained using this method may only be reported if the presence of a cocaine related compound is indicated by another detection technique, such as immunoassay or scan-mode mass spectrometry.

A case may not be reported as positive if indicated to be present below the LOD or LOQ for this assay.

### Precautions and Notes

Methanol must not be used to rinse the autoinjector syringe because it hydrolyzes the derivatives formed in this procedure. Anhydrous ethyl acetate should be used instead.

### Preparation of Materials

#### *0.1M Phosphate buffer:*

Dissolve 0.17 g Na<sub>2</sub>HPO<sub>4</sub> and 1.214 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O in 80 mL of deionized water. Dilute to 100 mL with deionized water. Adjust pH to 6.0±0.1 with Na<sub>2</sub>HPO<sub>4</sub> (lowers pH) or NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (raises pH). This solution may be stored for one month if refrigerated.

#### *0.1M Hydrochloric acid:*

Slowly add 4.2 mL of concentrated hydrochloric acid to 400 mL. Dilute to 500 mL with deionized water. This solution may be stored for six months at room temperature.

#### *Elution solvent:*

Mix 68 mL ethyl acetate, 28 mL methanol, and 4 mL ammonium hydroxide. This solution must be made daily for use.

## 6.7.14: ELISA Drug Screening

### Principle

**Enzyme Linked Immunosorbent Assay (ELISA)** is a competitive-binding immunoassay technique. It is used to indicate the presence or absence of a member of a class of drugs targeted by an antibody which binds preferentially with members of that class. This is determined by the response of the assay to a sample of unknown composition as compared to the response of a positive control of known composition.

Drug-class-specific antibodies coat the interior surfaces of the well of a 96-well plate. Each drug class has its own antibody and its own dedicated antibody coating. Sample is added to an microtiter well coated with this antibody and any drug in the sample will bind to the appropriate antibody binding sites. Then a combination enzyme conjugate is added, containing drugs labeled with horseradish peroxidase. The labeled drugs in the enzyme conjugate bind to the remaining antibody binding sites. After a reaction and equilibration period, the wells are emptied of liquid. When a chromogenic solution (**tetramethylbenzidine (TMB)**) is added, a color is produced in each well by the reaction of the TMB with the antibody-bound enzyme conjugate. The absorbance of the color in each well is proportional to the amount of labeled drug from the enzyme conjugate that is bound to the antibody binding sites. A stop solution (3N hydrochloric acid) is added to each well and the absorbance of each well at 450 nm is determined with a plate reader. The absorbance is inversely proportional to the amount of drug in the original sample.

### Specimens

This method is applicable with all specimen types, provided that the standards and controls are prepared in the appropriate matrix.

### Chemicals and Reagents

Millipore or reverse-osmosis purified water  
Enzyme conjugates (kit specific)  
TMB solution  
Stop solution

### Controls

Cutoff control  
Negative control

### Instrumentation

Thermo Multiskan 355 absorbance reader or equivalent

### Equipment

8-channel micropipette  
Single channel micropipette  
Single channel automated pipette  
Micropipette solvent troughs  
Pipette tips  
ELISA plates (kit specific)

### Instrument Conditions

Measurement mode: Absorbance  
Measurement wavelength: 450 nm

Reference wavelength: 650 nm  
Dual wavelength mode: Difference  
Reading mode: Accuracy  
Unit: OD

### Procedure

1. Place the appropriate test strips in a 96-well plate holder.
2. Dispense 30 µL of each sample or standard into the appropriate well.
3. Add 75 µL of enzyme conjugate reagent into each well.
4. Tap the plate gently approximately ten times to mix.
5. Incubate the plate at room temperature for approximately thirty minutes.
6. Remove the liquid from each well by inverting and flicking the plate.
7. Rinse the wells several times with cold tap water.
8. Fill each well with rinse solution, and then invert the plate to remove the liquid from each well.
9. Rap the inverted plate on dry toweling to ensure that the wells contain no residual liquid.
10. Add 100 µL of TMB chromogenic solution to each well.
11. Tap the plate gently to mix.
12. Incubate at room temperature for approximately fifteen minutes.
13. Add 50 µL of stop solution to each well.
14. Tap the plate gently to mix.
15. Read the plate on a microplate reader at 450 nm. Plates must be read within five minutes of the previous step.

### Quality Assurance

Controls should be made in the same matrix as the specimens, if possible. Matrix effects can be pronounced in ELISA. A cutoff control and a negative control are required on each plate.

The plate reader cannot read an absorbance greater than 4.0. If absorbances approach or reach this value, the second incubation may be shortened to decrease the absorbance. An absorbance greater than 4.0 in a matrix blank does not necessitate rerunning the assay.

The blank absorbance reading must be greater than 1.0 for each test.

The mean of the ODs of the two case aliquots is compared to the calibrator to determine whether the specimen is positive. Widely disparate ODs from the same source warrants further investigation.

### Precautions and Notes

Protect the TMB solution from light and heat. If the solution has a blue tint it is unsuitable for use and should be discarded.

Ensure that the conjugate lot being used has been tested with the test strips being used, as indicated on the exterior of the plate packaging and that plates as well as conjugates are not expired.

Once opened, plates should be kept sealed in the original package and stored in a dry place.

## 6.7.15: Differentiation of Phentermine, Methamphetamine, Amphetamine, MDMA, and Pseudoephedrine by LC-MS-MS

### Principle

This method uses liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) to detect and differentiate amphetamine, methamphetamine, phentermine, ephedrine/pseudoephedrine and 3,4-methylenedioxymethamphetamine (MDMA). It does this by monitoring ion transitions characteristic of each compound and unshared by coeluting members of the drug class.

### Specimens

This method is applicable to blood, urine, and other matrices. Controls and blanks should be made in the applicable matrix if possible. A 1 mL aliquot is normally used, but other specimen sizes may be applicable depending on matrix and concentration.

### Chemicals and Reagents

Sodium hydroxide  
Ammonium formate  
Concentrated formic acid  
Deionized water  
Methanol, HPLC grade  
n-Butyl chloride

### Reagents

0.1 N Formic acid  
5 N Sodium hydroxide  
Formate buffer mobile phase

### Controls

Amphetamine certified standard, 1 mg/mL  
Methamphetamine certified standard, 1 mg/mL  
Phentermine certified standard, 1 mg/mL  
Ephedrine certified standard, 1 mg/mL  
3,4-Methylenedioxymethamphetamine certified standard, 1 mg/mL

### Equipment

15 mL screw cap centrifuge tubes  
Pipets and pipettors  
Tube rotator  
Centrifuge  
Autosampler vials with inserts and crimp-on caps with rubber septa  
Crimper  
Vortex mixer

### Instrumentation

LC Pump:	Varian 210 or equivalent
Mass spectrometer:	Varian 1200L or equivalent
Autosampler:	Prostar 410 or equivalent

Column type:	Zorbax Eclipse XDB-C-18 or equivalent
Size:	3x150 mm
Particle size:	5 micron

## Instrument Conditions

Autosampler	
Mode:	Splitless
Injection volume:	5 µL

Mass Spectrometer	
Mode:	Positive
Source:	AP-ESI

Solvent			
Time (min)	% A	% B	Flow (mL/min)
0	80	20	0.2
10	80	20	0.2

LC-MS-MS Settings			
Analytes	Precursor Ion (M+H) <sup>+</sup>	Product Ion	Collision Energy (eV)
Amphetamine	136.1	119.0	-11
Methamphetamine	150.1	119.0	-9.5
Phentermine	150.1	133.0	-7.5
Ephedrine/Pseudoephedrine	166.1	148.0	-11
3,4-Methylenedioxymethamphetamine	194.0	163.0	-8

## Procedure

1. Aliquot samples and controls into labeled extraction tubes
2. Add 200 µL of 5 N NaOH to each tube and vortex well
3. Add 5 mL n-butyl chloride and rotate until fully mixed
4. Centrifuge until separated and remove the n-butyl chloride to a second tube
5. Add 150 µL of 0.1 N formic acid to each tube
6. Vortex 1 minute
7. Centrifuge tubes until separated
8. Recover approximately 100 µL of the lower formic acid layer into an autosampler vial
9. Analyze with LC-MS-MS

## Quality Assurance

These results need not be reported separately, but should be used to clarify what should be reported in the drug screen.

Other related drugs may be detected by running a known positive standard to determine the most appropriate transition to monitor.

Other column types and dimensions may be used, provided that both positive and negative controls are run and perform adequately.

## Precautions and Notes

Positive peaks must have a signal-to-noise ratio (S:N) of greater than ten. Negative samples may have no peak with the appropriate m/z or transition within 2% of the expected retention time with a S:N greater than 3.

Positive results must be compared against a positive control at a reasonable concentration.

## Preparation of Materials

5 N Sodium hydroxide:

Slowly add 100 g of sodium hydroxide to 500 mL of deionized water, stirring gently. Take care that the solution does not become too hot.

0.1 N Formic acid:

Add 425  $\mu$ L concentrated formic acid to 100 mL water and mix well.

Formate buffer mobile phase:

Add 0.441 g of ammonium formate and 350  $\mu$ L of concentrated formic acid to 700 mL of deionized water in a class A 1 L volumetric flask and make up to 1 liter with methanol.

COPY

## ***Standard Operating Procedures - Other Methods***

### **6.7.16: Analytical Calibration Curve Preparation**

A calibration curve must be made of at least three points of varying concentration designed to encompass the concentration range of interest. Note that the specimen sample aliquot size and dilution factor may be varied in order to bring the measured concentration into the range of the calibration curve.

The samples used to generate the calibration curve must be extracted or otherwise prepared in the same manner as case specimens, as far as is possible and appropriate.

The standard number or lot number of each control used to generate the calibration curve (including the internal standard) must be recorded. The location and/or identity of the original data file used to generate the calibration curve must be recorded, if known. A curve evaluation worksheet is provided (TOX-FORM-09) which may be used to record this information.

Calibration curves should be generated using a least-square or other well-accepted curve fitting algorithm. The origin will not be used as a data point. The calibration curve will not be forced through the origin.

### **6.7.17: Calibration Curve Evaluation**

Once generated, the calibration curve is evaluated according to the following criteria:

- The correlation coefficient must be at least 0.990.
- The measured value of each curve point must be within 20% of its known value, with the exception of the lowest curve point, which may be up to 30% off.

If the correlation coefficient is less than 0.990 then the analyst should look for "outliers", where the curve point is uncharacteristically off of the curve. The integration of this point (both analyte and internal standard) should be checked to see if it is integrated differently from the other standards. If the integrated peak is integrated in a different manner than the other peaks then this integration may be manually corrected or the integration parameters may be changed to make the integration more consistent between specimens. If the integration parameters are changed, then all points must be re-integrated and a new curve constructed. If the integration is consistent with the integration of the other curve points and the outlier is still significantly off of an otherwise linear curve, then this point may be discarded.

If the measured value of a curve point differs too much from the known value of that point, then the integration should be checked as above. If the integration is consistent with the integration of the other curve points, then this point may be discarded.

Only one point should be discarded for these reasons. More than one outlier may indicate that the curve is not valid and should not be used to generate quantitative results.

If the lowest point on the curve does not have an adequate response to meet quality control criteria, then this point may be discarded. The detection limit of the calibration curve is concomitantly raised to the lowest point that meets the quality control criteria.

In a selected ion monitoring (SIM) quantitation, the ratio of the qualifier ion(s) to the quantitation ion is measured for each peak. If this ratio differs by more than 20% (or the amount specified in the method) relative to a measured value from a known standard, then the qualifiers fail and the point is invalid. In this case the integration should be checked as above for each ion to determine whether the fault is with an inconsistent integration. If so, then the ion(s) may be manually reintegrated, the integration parameters may be changed, or a new standard may be chosen upon which to base the qualifier ratio calculation.

All dilutions of certified standards will be made using measured volumetric amounts.

The amount of internal standard added to a standard or control should be within an order of magnitude of the amount of analyte in that standard or control.

After the generation of an acceptable calibration curve, all control samples are quantitated against the curve. The measured value of the control samples must not be more than 20% from their known value, using the formula:

$$\text{Percent difference} = ((\text{measured value} - \text{known value}) / \text{known value}) \times 100$$

If the measured values of a control differs more than 20% from its known value, further investigation to determine the source of the discrepancy and appropriate action to correct it is warranted. This normally requires the extraction and analysis of a new control from a third source, if possible. If it is demonstrated that the curve is in error then a new calibration curve must be constructed. If it is demonstrated that the error is confined to the original control sample(s) then the cases may be quantitated against the curve. If a problem in the extraction is demonstrated, then re-extraction of the samples is necessary.

If a calibration curve cannot be generated that meets these criteria of acceptability, then this calibration curve is invalid and may not be used to generate quantitative results.

A curve evaluation worksheet is provided (TOX-FORM-09) which may be used to record this information. This information will be kept for every calibration curve used to generate quantitative results to ensure that the results are based on data of sufficient quality.



## Section 7: Maintenance

The instruments and equipment in the Forensic Toxicology Section will be routinely maintained by the section employees when possible. Major repairs may be performed by a service engineer, preferably from the original equipment manufacturer.

A maintenance log is located by each instrument to contain a record of all routine and non-routine maintenance performed on that instrument. It must contain a description of the maintenance, the date the maintenance was performed, and the identity of the person(s) performing the maintenance. It also records the method by which it is verified that the instrument is in proper working order. If this is by the analysis of controls, then the location of those controls must be specified.

Designated instruments require the maintenance of a QC logbook which includes the following:

- Outline of normal operating parameters (e.g. oven program, gas flow rate)
- Record of all calibration and quality control checks
- Record of all maintenance performed on the instrument

If an instrument is removed from service pending repair, a record of the repair and of the proper functioning of the instrument must be made before the instrument is placed back in service.

The requirements for maintenance vary according to instrument type. The general requirements are:

### Gas Chromatographs (GCs)

The septum and injection liner should be replaced weekly or as needed. Any decrease in the quality of the chromatography should be noted and appropriate documented action taken to correct the problem.

The solvent wash bottles should be rinsed and filled with the appropriate solvent as needed. The waste bottles should be rinsed and emptied into waste containers.

### Gas Chromatograph-Mass Spectrometers (GC-MSs)

The gas chromatograph portion of this instrument is maintained as listed above.

The GC-MS will be auto-tuned at least weekly, if used, and should be tuned before each sequence is run. The GC-MS will be auto-tuned prior to running a selected ion monitoring (SIM) method. All tune reports should be maintained in a logbook. The autotune uses the 69, 219, and 502 m/z produced by the calibration compound PFTBA to optimize various parameters for the Mass Selective Detector. After the autotune report has printed, the chemist will assess the calibration by examining the autotune report for the following items:

1. If the abundance of any peak(s) below 69 m/z (e.g. 18[water], 28[nitrogen], 32[oxygen]) are >20%, relative to the abundance of the 69 m/z peak. The water peak at m/z 18 must be less than 10%, and is optimally much less than 5%. Any significant peak at m/z 28 is indicative of a nitrogen contamination from a leak or from a contaminated gas cylinder
2. If the EM voltage is > 2500

If the tune report indicates that the tune is acceptable, the analyst checking the tune report will initial the tune report.

If either of these conditions exist, the instrument is not in proper working condition and must be removed from service until it has been repaired and has passed calibration. A record of the

remediation and proper functioning of the instrument, usually in the form of a successful tune, must be recorded.

Other maintenance is performed on an as needed basis. When the GC-MS has been removed from service to clean the source or replace the filaments, maintenance should be performed on the following items, as needed:

1. The source should be cleaned following manufacturer-recommended procedures
2. The filaments should be replaced
3. The diffusion pump oil should be inspected and replaced if necessary
4. The fore-line pump oil should be checked and filled or replaced if necessary
5. The vent line should be rinsed with methanol
6. The vent line trap should be inspected and replaced if necessary
7. The gold seal should be inspected and replaced if necessary

### **CO-Oximeter**

The CO-Oximeter uses control samples supplied by the manufacturer to ensure that the instrument is responding properly. These analytical results from these controls must fall within a range of values supplied with the control samples. A control is run with each batch of casework.

### **AxSYM**

The AxSYM is maintained according to the manufacturer's specifications. Maintenance is performed monthly, weekly, and daily (if the AxSYM is used). Control samples are run daily (if the AxSYM is used) to ensure that the instrument is responding within specifications. Any significant repair should be performed by a company representative.

### **Balances**

The calibration of each balance will be checked daily (if used) with traceable standards before any measurements are made. If the calibration is off then the balance must be recalibrated. The acceptability range for an analytical balance using a 100 gram calibration mass is 99.9998-100.0002 grams. The acceptability range for a top loading balance using a 100 gram calibration mass is 99.9-100.1 grams. The procedure for calibration will vary from balance to balance.

### **Pipettes**

Pipettes will be checked each calendar year and repaired if necessary, typically by the pipette manufacturer.

## **Section 8: Proficiency Testing Program**

The Forensic Toxicology Section policies regarding audits conform to the lab-wide policies and may be found in the lab-wide quality manual. Additions and clarifications to the lab-wide policies are listed here.

In the Forensic Toxicology Section an external proficiency test must be completed annually in both the analysis of specimens for alcohol content and the analysis of specimens for drug content. One of these two external proficiencies must be an approved test from an ASCLD/LAB approved provider. The list of approved tests and providers can be obtained from ASCLD/LAB.

Proficiency tests should be run as identically as possible to casework, including technical and peer review. There are two main exceptions to this. First, proficiency test providers may have additional requirements regarding testing and/or reporting that we must follow. Second, proficiencies are not subject to policies put into place for efficiency or expediency of casework. For example, if an immunoassay is positive for a class of compounds, we must attempt to detect all of the members of that class to the extent of our ability even if we would not normally continue to test for members of that class once one had been confirmed.

In addition to proficiency testing, case re-examination or blind analysis may be performed in the Forensic Toxicology Section. This allows the laboratory to demonstrate that proficiency samples are treated in the same manner as cases.

Case re-examination can be achieved in the Forensic Toxicology Section in one of two ways. First, a completed case may be reassigned to a second analyst for reanalysis. The first analyst must not have been previously aware that the case will be reanalyzed. Second, duplicate samples may be submitted and analyzed concurrently by two analysts if the two analysts are not aware of the duplicate analysis.

Blind analysis can be achieved by the submission of a sample of known composition. The sample is submitted as a regular case and the analysis must be performed without the analyst being aware that the sample is a blind sample.

### ***8.1: Sources of testing***

Among approved external proficiency test providers are the College of American Pathologists (FTC proficiency) and Collaborative Testing Services. A list of all approved tests from approved providers may be obtained from ASCLD/LAB.

Internal proficiency testing is acceptable if approved external proficiency tests have been completed. The Chief Forensic Toxicologist or a designee prepares a sample representative of casework. Twice the required amount of specimen is prepared for analysis. Half is given to the analyst and half retained for reanalysis, if necessary. The analyst is told the type of analysis required. After analysis, technical review, and administrative review, the Chief Forensic Toxicologist or section Quality Manager will review and evaluate the case record.

### ***8.2: Evaluation of results***

External proficiency test providers generally supply an evaluation of the results of their proficiency test. If no evaluation is provided, then the results are evaluated on the basis of acceptability in the field as a whole.

For internal proficiency tests the standard is whether the analytical results are within the expected error for the analysis performed (e.g. within 20% for quantitations).

COPY

## Section 9: Case Records

The Forensic Toxicology Section policies regarding case records (including reporting, release of information, case review, and corrective actions) conform to the lab-wide policies and may be found in the lab-wide quality manual. Additions and clarifications to the lab-wide policies are listed here.

### 9.1: Case File Storage

Recent paper case files will be destroyed after having been entered into the LIMS. Older paper case files are stored in the Forensic Toxicology Section, the file rooms in the annex, or off-site storage.

### 9.2: Data Retention

Where possible, data will be backed up to a removable storage medium such as a recordable CD (CDR) or DVD (e.g. DVD+R) before deletion. If this is not feasible for technical reasons, then data may be deleted without back-up if necessary.

All data should be backed up in such a way to be able to identify the source of each datafile.

#### ***Arkansas Code 12-12-312. Records confidential and privileged - Exception - Release.***

*(a)(1)(A)(i) The records, files, and information kept, obtained, or retained by the State Crime Laboratory under the provisions of this subchapter shall be privileged and confidential.*

*(ii) They shall be released only under and by the direction of a court of competent jurisdiction, the prosecuting attorney having criminal jurisdiction over the case, or the public defender appointed or assigned to the case.*

*(B)(i) Provided, that nothing in this section shall be construed to diminish the right of a defendant or his or her attorney to full access to all records pertaining to the case.*

*(ii) Provided further, that the State Crime Laboratory shall disclose to a defendant or his or her attorney all evidence in the defendant's case.*

*(2) However, a full report of the facts developed by the State Medical Examiner or his or her assistants shall be promptly filed with the law enforcement agencies, coroner, and prosecuting attorney of the jurisdiction in which the death occurred.*

*(b) The State Crime Laboratory Board shall promulgate rules and regulations not contrary to law regarding the release of reports and information by the staff of the State Crime Laboratory.*

*(c) All records, files, and information obtained or developed by the State Crime Laboratory pertaining to a capital offense committed by a defendant who is subsequently sentenced to death for the commission of that offense shall be preserved and retained until the defendant's execution.*

*History. Acts 1969, No. 321, §11; 1979, No. 864, §16; A.S.A. 1947, §§42-621, 42-1218; Acts 1993, No. 1304, §1; 1999, No. 519, §1; 2001, No. 211, §1; 2001, No. 917, §1.*

## **Section 10: Testimony Review**

The Forensic Toxicology Section policies regarding testimony review conform to the lab-wide policies and may be found in the lab-wide quality manual.

COPY

## **Section 11: Audits**

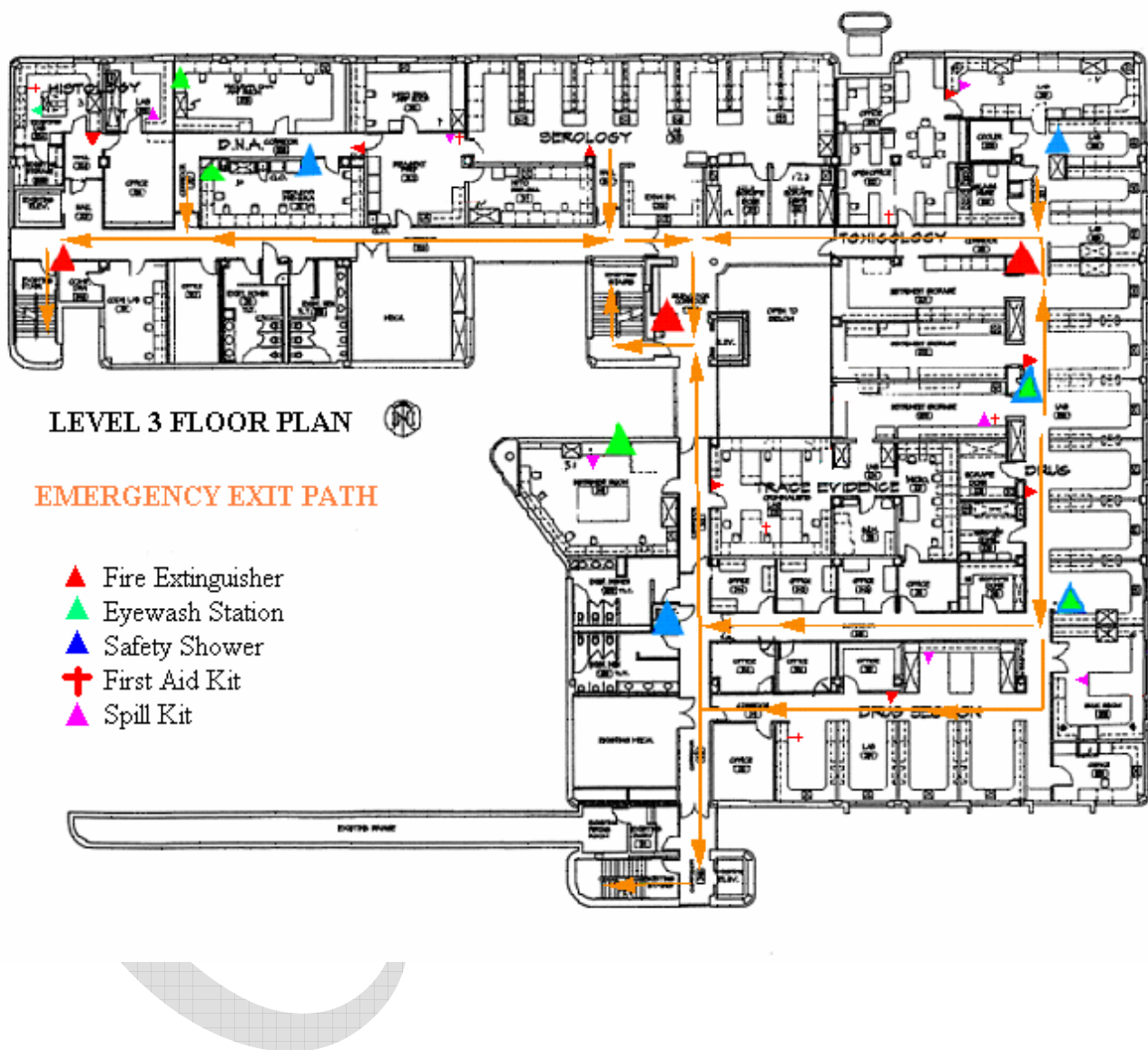
The Forensic Toxicology Section policies regarding audits conform to the lab-wide policies and may be found in the lab-wide quality manual.

COPY

## Section 12: Health and Safety

The Forensic Toxicology Section policies regarding health and safety conform to the lab-wide policies and may be found in the lab-wide health and safety manual. Additions and clarifications to the lab-wide policies are listed here.

The evacuation route from the Forensic Toxicology Section is through the central stairwell. If this path is blocked or otherwise unavailable, the next shortest safe route should be taken.





## **Section 13: Complaints**

The Forensic Toxicology Section policies regarding case records conform to the lab-wide policies and may be found in the lab-wide quality manual and/or personnel handbook.

COPY

## Section 14: Miscellaneous

### 14.1: Literature Review

A review of current literature is encouraged. To this end, articles from journals of interest are made available in the Forensic Toxicology Section in printed form. A signature sheet is kept for each article to document the reading of articles. Additionally, many journal articles are maintained in an electronic format on a shared drive accessible to all members of the Forensic Toxicology Section.

The Arkansas State Crime Laboratory maintains subscriptions to academic journals. These journals are kept in various locations in the laboratory. Analysts are encouraged to read these journals and distribute any articles of interest. Articles of interest may be presented at section meetings.

### 14.2: Glossary

- *Blind Test*: A test in which the analyst is unaware of the test nature of the sample at the time of analysis.
- *Blind Trial*: An internally generated sample whose composition is unknown to the analyst.
- *Certified Standard*: A primary standard solution with an externally certified concentration
- *Competency Test*: A test to determine the analytical competence of analyst/examiner trainees prior to performing independent casework. A competency test should include a written test, internal proficiency tests, and moot court (for new employees or employee training in new sub-disciplines).
- *Concordance Testing*: testing which is an external procurement or exchange of blind and reference samples with another competent laboratory.
- *Confirmed*: The presence if the indicated compound(s) has been shown in two different specimen types, aliquots of the same specimen, or by two analytical techniques based on different principles
- *Control*: Sample which verifies procedure is working as expected.
  - *Positive control*: A control for which a positive response is expected.
  - *Cutoff control*: A control which is used to determine whether an assay is considered to be Positive or Negative by comparing the response of the unknown to the response of the cutoff control. It is a subclass of positive controls.
  - *Negative control*: A control for which a negative response is expected.
- *Corroborated*: The quantitative amount of the indicated compound has been reproduced in more than one sample within the quality control constraints of the procedure.
- *Detected*: The testing has produced a response consistent with the presence of the indicated compound(s) and inconsistent with their absence.
- *External Proficiency Test*: A test to evaluate the competence of analysts, technical support personnel, and the quality performance of the laboratory conducted by an independent agency.
- *Internal Proficiency Test*: A test to evaluate the competence of analysts, technical support personnel, and the quality performance of the laboratory conducted by the laboratory itself.
- *Lethal*: At a concentration where death may occur as a direct result of the presence of the drug.
- *Normal*: At a concentration consistent with average environmental exposure
- *Negative*: The testing has produced a response insufficient to indicate the presence of the analyte(s) above a threshold amount
- *Not detected/none detected*: The indicated compound(s) have not been detected, but would be expected to if present in significant amounts.
- *Per se limit*: A value above which a specific conclusion is legally warranted (e.g. 0.08 g% blood ethanol value indicates legal intoxication)
- *Positive*: The testing has produced a response sufficient to indicate the presence of the analyte(s) above a threshold amount
- *Present*: The indicated compound(s) have been detected.

- *Reagent*: Any substance used for its chemical or biological activity.
- *Re-examination Test*: A test in which a previously examined sample is re-analyzed by a different analyst/examiner.
- *Split Samples*: A homogeneous sample portioned out for separate analysis.
- *Standard*: Substance of known quantity and/or quality.
- *Therapeutic*: At a concentration where a drug produces its intended effect.
- *Toxic*: At an increased concentration where deleterious effects may appear in addition to the intended effects of the drug.
- *Uncertified Standard*: A standard solution which does not have an externally certified concentration

### **14.3: List of Standard Abbreviations**

These abbreviations are standard abbreviations and may be used in case files without further explanation. Other abbreviations may be used if they can be unambiguously understood by an external reviewer.

- |                            |   |
|----------------------------|---|
| • A: acid extraction       | • ME: manila envelope                   |
| • AB: antemortem blood     | • mip: marked in part                   |
| • AF: Abdominal fluid      | • MS: muscle                            |
| • AM: antemortem           | • ND: none detected                     |
| • B: base extraction       | • NDD: no drugs detected                |
| • BBA: blood blank acid    | • neg: negative                         |
| • ABK: acid blank          | • NL: not labeled                       |
| • BBB: base blood blank    | • N/L: not labeled                      |
| • BBK: base blank          | • p: page                               |
| • BBU: urine blank         | • PB: peripheral blood                  |
| • BK: blank                | • pg: page                              |
| • BL: bile                 | • PL: pleural fluid                     |
| • BLK: blank               | • pls: plastic                          |
| • BR: brain                | • pos: positive                         |
| • cont: containing         | • PR: pericardial fluid                 |
| • cont'd: continued        | • QNS: quantity not sufficient          |
| • CS: cerebrospinal fluid  | • SB: stat blood                        |
| • CSF: cerebrospinal fluid | • SD: subdural                          |
| • CV: cavity fluid         | • STC: said to contain                  |
| • GS: gastric contents     | • UB: unknown blood                     |
| • HB: heart blood          | • UR: urine                             |
| • hs: heat-sealed          | • VT: vitreous humor                    |
| • KD: kidney               | • (curled arrow): containing            |
| • LN: lung                 | • (p with dot above it): marked in part |
| • LV: liver                |   |

End of Document