FORENSIC TOXICOLOGY

QUALITY MANUAL

DIRECTOR: KERMIT B. CHANNELL, II

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1 SCOPE

This manual follows the requirements specified by ANSI-ASQ National Accreditation Board (ANAB), which are based on the ISO/IEC 17025:2017 standards and the 2017 ANAB ISO/IEC 17025:2017 — Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125).

The ASCL Quality Manual (ASCL-DOC-01) outlines the policies and procedures under which the laboratory operates. This manual acts as a set of supplemental policies and procedures required to competently perform testing in the Forensic Toxicology Discipline at the Arkansas State Crime Laboratory.

When the section policy does not differ from the lab wide policy in any significant manner, the reader will be referred to the *ASCL-DOC-01 Quality Manual* for the policy. Where there are additional policies and/or procedures, clarifications, or another basis for further information, then that will be included in this document.

The *Forensic Toxicology Quality Manual* is written specifically for the analysts working in the Toxicology Section and performing analysis in the following areas:

- Qualitative Determination
- Quantitative Measurement

1.1 INTERNATIONAL STANDARD: GENERAL REQUIREMENTS

See *ASCL-DOC-01 Quality Manual*.

1.2 INTERNATIONAL STANDARD: SCOPE

See *ASCL-DOC-01 Quality Manual*.

1.2.1 ANAB PROGRAM

See *ASCL-DOC-01 Quality Manual*.

2 NORMATIVE REFERENCES

The Forensic Toxicology section follows applicable references listed in *ASCL-DOC-01 Quality Manual*. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

Additional references include:

- SOFT/AAFS Forensic Toxicology Laboratory Guidelines (2006 version)
- Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology (Published 01 September, 2013)
- Standard Practices for Method Validation in Forensic Toxicology (ANSI/ASB Standard 036, First Edition 2019)
- *ASCL Personnel Handbook (ASCL-DOC-02)*
- *ASCL Health and Safety Manual (ASCL-DOC-08)*
- *Forensic Toxicology Section Training Manual (TOX-DOC-02)*

These manuals will be reviewed annually and revised as needed. They are available at all locations where they are essential to the effective functioning of the laboratory (i.e., the Forensic Toxicology Section).

3 TERMS AND DEFINITIONS

Additions to *ASCL-DOC-01 Quality Manual* are listed below.

ACCURACY

The closeness of agreement between a measured quantity value and the true quantity value of a measurand, usually reported as a percent difference. The term *bias* may also be used to describe accuracy.

BLANK MATRIX SAMPLE

A biological fluid or tissue sample (or synthetic substitute) without target analyte or internal standard.

CALIBRATION MODEL

A mathematical model that demonstrates the relationship between the concentration of an analyte and the corresponding instrument response.

CALIBRATOR

A solution that is used to calibrate assays. This solution is either purchased or prepared from a reference material.

CARRYOVER

The appearance of unintended analyte signal in samples after the analysis of a positive sample.

CERTIFIED STANDARD

A primary standard solution with an externally certified concentration.

CHEMICAL

A substance or compound used for its constant chemical composition or characteristic properties. *Examples: Acidic or basic solutions.*

CONCORDANCE TESTING

Testing which is an external procurement or exchange of blind and reference samples with another competent laboratory.

CONFIRMED

The presence of 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

A solution that is either purchased or prepared from a reference material that is separate from calibrators. A control is utilized to ensure that a method and/or instrument are working as expected. *Examples: Positive, negative, and cutoff controls*

CORROBORATION/CORROBORATED RESULT

A result which has been demonstrated in more than one specimen or testing event, within the quality control constraints of the method. A corroboration may be qualitative or quantitative.

CUTOFF CONTROL

A control which is used to determine whether an assay is considered to be positive or negative by comparison of the response of the unknown to the response of the cutoff control. It is a subclass of positive controls.

DECISION POINT

An administratively defined cutoff or concentration that is at or above the method's limit of detection or limit of quantitation and is used to discriminate between positive and negative results.

DETECTED

The testing has produced a response consistent with the presence of the indicated compound(s) and inconsistent with their absence.

DILUTION INTEGRITY

A determination that accuracy and precision are not significantly impacted when a sample is diluted.

FLUID

Any biological liquid specimen that is typically pipetted for analysis.

FORTIFIED BLANK MATRIX SAMPLE

A blank matrix sample spiked with target analyte and/or internal standard, using reference materials.

INTERFERENCES

Non-targeted analytes (e.g., matrix components, other drugs and metabolites, internal standard, impurities) which may affect the ability to detect, identify, or quantitate a targeted analyte.

IONIZATION SUPPRESSION/ENHANCEMENT

Direct or indirect alteration of, or interference with, instrument response due to the presence of co-eluting compounds.

ISSUING AUTHORITY

Personnel that are authorized to post the approved controlled documents in Qualtrax.

LETHAL

At a concentration where death may occur as a direct result of the presence of the drug.

LIMIT OF DETECTION

An estimate of the lowest concentration of an analyte in a sample that can be reliably detected or identified, but not necessarily quantitated, by the analytical method. This is also referred to as the "detection limit", or "LOD".

LIMIT OF QUANTITATION

An estimate of the lowest concentration of an analyte in a sample that can be reliably differentiated from blank matrix and measured with acceptable accuracy and precision. This is also referred to as the "quantitation limit", or "LOQ".

LIMIT OF REPORTING

A concentration (or response) below which an analyte may remain unreported, even though it has been detected. This is also referred to as the "reporting limit", or "LOR".

LINEAR RANGE

The concentration range within which it has been demonstrated that instrument response is proportional to the value of the measurand. This is typically the range bounded by the lowest and highest calibrator. Also called the "working range".

NEGATIVE

The testing has produced a response insufficient to indicate the presence of the analyte(s) above a threshold amount.

NEGATIVE CONTROL

A control for which a negative response is expected.

NORMAL

At a concentration consistent with expected environmental exposure.

NOT DETECTED/NONE DETECTED

The indicated compound(s) have not been detected, but would be expected to if present in significant amounts.

NULL HYPOTHESIS

The default condition, which must be disproven in order to accept the alternative hypothesis. *Example: the null hypothesis in forensic toxicology is that an analyte is not present. This must be disproven in order to accept the alternative hypothesis, which is that the analyte is present.*

PER SE

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. Note that "positive" refers to the assay response, not the presence of the targeted analyte.

POSITIVE CONTROL

A control for which a positive response is expected.

PRECISION

The measure of the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample. It is expressed numerically as imprecision.

PRESENT

The indicated analyte has been detected.

QUALTRAX

An intranet framework which provides a secure repository of controlled documents and forms, workflows, and additional functionality.

REAGENT

A substance used because of its known chemical or biological activity. *Examples: TMB solution.*

RECOVERY

The extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.

ROBUSTNESS

The measure of an analytical method's resistance to result changes when minor deviations are made in the experimental conditions described in the method. It provides an indication of the method's reliability given the small changes that are expected to occur during routine use.

SPLIT SAMPLES

A homogeneous sample portioned out for separate analysis.

STABILITY

The analyte's resistance to chemical change in a matrix under specific conditions for given time intervals.

STANDARD

A substance of known quantity and/or quality.

SUBTHERAPEUTIC

Below a concentration where a drug produces its intended effect.

THERAPEUTIC

At a concentration where a drug produces its intended effect.

TISSUE

Any solid biological specimen that is generally weighed (massed) for analysis.

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.

4 GENERAL REQUIREMENTS

4.1 IMPARTIALITY

See *ASCL-DOC-01 Quality Manual*.

4.1.1 GENERAL

The Forensic Toxicology Section shares the policies and procedures outlined in §4.1.1 of the lab wide quality manual as applicable.

In addition to the items listed in §5.2.9.1 of this manual, the Chief Forensic Toxicologist has the authority required to:

- Maintain, implement, and improve the management system
- Identify departures from the management system
- Initiate actions to prevent or minimize such departures

The Forensic Toxicology Section Quality Manager has the authority required to:

- Monitor compliance with the quality system through monitoring of activities and evaluation of records
- Maintain quality records

The Forensic Toxicology Section Safety Manager has the authority required to:

- Monitor compliance with the health and safety system
- Maintain health and safety records

4.1.2 PERSONNEL

The organizational structure of the Forensic Toxicology Section conforms to that contained in the lab wide quality manual (*ASCL-DOC-01 Quality Manual*). Within the section, each Forensic Toxicologist, Forensic Technician, and the Technical Leader reports directly to the Chief Forensic Toxicologist.

The chain of command must be followed whenever possible. All concerns and grievances must first be addressed with the immediate supervisor-skipping organizational levels is prohibited.

If the Chief Forensic Toxicologist will be absent from the laboratory for three or more days, then a deputy will be appointed, and this appointment will be communicated to all affected personnel.

4.1.3 FISCAL

See *ASCL-DOC-01 Quality Manual*.

4.1.3.1 ETHICAL PRACTICE

See *ASCL-DOC-01 Quality Manual*.

4.1.4 RISKS TO IMPARTIALITY

Risks are evaluated whenever necessitated by a change to lab policy or by external actions/situations.

4.1.5 ACTIONS TAKEN IN RESPONSE TO RISK

If a risk to the impartiality of the ASCL is identified, the actions taken to minimize or eliminate the risk will be recorded.

4.2 CONFIDENTIALITY

4.2.1 STATUTE

Case information at the ASCL is controlled by state statute (§12-12-312). This includes case information either obtained or created during the performance of laboratory activities.

Records, files, and information kept, obtained, or retained by the ASCL are privileged and confidential. However, the ASCL shall grant access to records pertaining to a defendant's criminal case to:

- the defendant,
- the public defender or other attorney of record for the defendant,
- the prosecuting attorney or deputy prosecuting attorney having jurisdiction over the criminal case, and
- to another party at the direction of
  - a court of competent jurisdiction, or
  - the prosecuting attorney having criminal jurisdiction over the case

Customer agencies that have made the necessary arrangements with the ASCL are granted secure access to JusticeTrax iResults, where they may check on the status of their laboratory requests and view completed reports for their agency. JusticeTrax access is secured by username/password.

Stat results may be released to the Medical Examiner's Office after a technical review has been performed and documented in the case notes.

4.2.2 THIRD-PARTY RELEASE

See *ASCL-DOC-01 Quality Manual*.

4.2.3 THIRD-PARTY SOURCE

See *ASCL-DOC-01 Quality Manual*.

4.2.4 SCOPE OF CONFIDENTIALITY

See *ASCL-DOC-01 Quality Manual*.

5 STRUCTURAL REQUIREMENTS

5.1 ESTABLISHMENT

Act 517 of 1977 established the Arkansas State Crime Laboratory (ASCL) via A. C. A. § 12-12-301.

5.2 MANAGEMENT

The Arkansas State Crime Laboratory is managed by the Director, who has overall responsibility for the laboratory.

For §5.2.1 – 5.2.6 See *ASCL-DOC-01 Quality Manual*.

5.2.7 OTHER STAFF (FORENSIC TOXICOLOGY STAFF)

5.2.7.1 CHIEF FORENSIC TOXICOLOGIST

QUALIFICATIONS

The position requires the formal education equivalent of a bachelor's degree chemistry, biology, or a related field, five years' experience in a chemical laboratory (including two years as a forensic toxicologist), and 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 Director and the Assistant Director. The Chief Forensic Toxicologist, or a designee, will have appropriate technical training and experience in forensic toxicology.

AUTHORITIES AND RESPONSIBILITIES

The Chief Forensic Toxicologist is under administrative direction and is responsible for the activities of the Forensic Toxicology Section in Little Rock and satellite laboratories. The Chief Forensic Toxicologist has the overall responsibility for the technical operations and the provision of the resources needed to ensure the quality of the laboratory operations. The Chief Forensic Toxicologist will have the appropriate technical training and technical experience in the toxicology section.

The Chief Forensic Toxicologist will have regular contact with crime laboratory staff, frequent contact with law enforcement agencies and judicial officials, and limited contact with the public. The Chief Forensic Toxicologist ensures compliance with ANAB requirements by implementing lab wide policies and overseeing the section's quality assurance program.

- Supervises a technical staff of Forensic Toxicologists including interviewing applicants and recommending for hire, approving leave, making work assignments, training employees and evaluating the performance of employees

- Assists with developing laboratory policies and procedures, develops short and long-range operational plans for the forensic toxicology section, monitors operational activities by conducting staff meetings to disseminate information and reviewing and approving reports and compiles and submits statistical reports
- Performs qualitative and quantitative forensic toxicology analysis on evidence received from law enforcement agencies and Medical Examiners for the presence and levels of alcohol, drugs and other toxic substances
- Presents expert forensic testimony in court on the analytical methodology used to analyze evidence and analysis results, supervises pretrial conferences, and provides consultation to law enforcement and judicial officials on evidence collection and preservation method
- Compiles and interprets data obtained from analytical instruments, reviews and approves scientific forensic reports of section toxicologists, and writes conclusive scientific forensic reports
- Conducts research studies and validates new forensic analytical procedures, reviews current scientific literature and attends and participates in meetings and seminars to keep abreast of new technologies and procedures in the field.
- Performs related responsibilities as required or assigned

5.2.7.2 FORENSIC TECHNICAL LEAD

QUALIFICATIONS

The Technical Leader must possess a baccalaureate or advanced degree in chemistry, biology, or biochemistry; five years' experience in a chemical laboratory (including two years as a Forensic Toxicologist), and one year in a leadership capacity. A master's or doctorate degree can be substituted for all or part of these basic requirements. The Technical Leader will have appropriate technical training and experience in Forensic Toxicology and validations.

AUTHORITES AND RESPONSIBILITIES

- Maintaining all equipment, reference standards, and materials within the Toxicology Section
- Maintaining and annually reviewing the Toxicology Section Quality Manual along with the Chief Forensic Toxicologist, as necessary
- Oversees the Toxicology training program for new employees
- Actively involved in Quality Assurance Concerns, including control charting QA/QCs
- Troubleshooting and repairing instrumentation as needed
- Updating Uncertainty of Measurement budgets annually
- Assists with developing laboratory policies and procedures, develops short and long-range operational plans for the Forensic Toxicology section
- Reviewing and approving reports and compiles and submits statistical reports
- Performs qualitative and quantitative forensic toxicology analysis on evidence received from Law Enforcement agencies and Medical Examiners for the presence and levels of alcohol, drugs and other toxic substances

- Presents expert forensic testimony in court on the analytical methodology used to analyze evidence and analyze results, supervises pretrial conferences, and provides consultation to Law Enforcement and judicial officials on evidence collection and preservation method
- Compiles and interprets data obtained from analytical instruments, reviews and approves scientific forensic reports of section toxicologists, and writes conclusive scientific forensic reports
- Conducts research studies and validates new forensic analytical procedures, reviews current scientific literature and attends and participates in meetings and seminars to keep abreast of new technologies and procedures in the field
- Performs related responsibilities as required or assigned

5.2.7.3 FORENSIC TOXICOLOGIST

QUALIFICATIONS

The Forensic Toxicologist must possess a baccalaureate or advanced degree in chemistry, biology, or a closely related field with knowledge of the principles and practices of chemistry, chemical analysis and laboratory equipment. Before performing casework, the forensic toxicologist will be required to successfully complete an internal training program that will include competency sample testing, written and oral examination, and a mock trial (this training program can be modified based on experience). This position is governed by state and federal laws and agency policy.

AUTHORITIES AND RESPONSIBILITIES

- Performs qualitative and quantitative forensic toxicology analysis on evidence received from law enforcement agencies and Medical Examiners for the presence and levels of alcohol, drugs and other toxic substances
- Present expert forensic testimony in court on the analytical methodology used to analyze evidence and obtain results
- Participate in pretrial conferences and provide consultation to law enforcement and judicial officials on evidence collection, preservation methods and analysis results.
- Verify the correct operation of scientific instruments and perform routine maintenance as needed. Prepare and verify reference materials and reagents according to established guidelines
- Review current scientific literature. Study and validate new forensic analytical procedures and modify new and/or old procedures as necessary
- Attend and participate in professional meetings and seminars to keep abreast of new technologies and methods in toxicology and chemistry
- Assist with training new laboratory staff in performing laboratory analysis.
- Perform related responsibilities as required or assigned

5.2.7.4 FORENSIC TECHNICIAN

QUALIFICATIONS

This position requires the formal education equivalent of a high school degree.

AUTHORITIES AND RESPONSIBILITIES

- Accession and organization of evidence submitted for Toxicology by law enforcement agencies within the state of Arkansas, as well as Medical Examiners in the Arkansas State Crime Laboratory
- Appears in state and federal courts to testify to the accession and chain of custody in legal criminal proceedings when necessary
- Perform related responsibilities as required or assigned

5.2.7.5 HEALTH AND SAFETY OFFICER

- Conducts monthly safety inspections and ensuring that proper practices and procedures are being followed in the section
- Maintains records of any safety incidents within the section
- Maintains a current copy of the section's SDSs
- Works with the lab wide Health and Safety Manager to seek ways to improve the safety program

5.3 SCOPE OF LABORATORY ACTIVITIES

The Forensic Toxicology Section carries out testing activities defined by its scope of accreditation which includes qualitative testing and quantitative testing.

5.4 NORMATIVE DOCUMENTS

See §2 for a list of normative documents used in the Forensic Toxicology Section.

5.4.1 USE OF ACCREDIATATION SYMBOLS

See *ASCL-DOC-01 Quality Manual*.

5.4.2 STATUTORY AUTHORITY

See *ASCL-DOC-01 Quality Manual*.

5.5 LABORATORY OPERATIONS

5.5.1 GENERAL

See *ASCL-DOC-01 Quality Manual*.

5.5.2 AUTHORITIES AND INTERRELATIONSHIPS

The organizational structure of the Forensic Toxicology Section conforms to that contained in the lab wide quality manual (*ASCL-DOC-01 Quality Manual*). Within the section, the Technical Lead, each Forensic Toxicologist, and each Forensic Toxicology Technician reports directly to the Chief Forensic Toxicologist.

5.5.3 QUALITY MANUAL

The purpose of the Forensic Toxicology Section Quality Manual is to document the policies and procedures of the analytical section. This document is readily available to all laboratory personnel via Qualtrax, and on the website to the public. This manual is reviewed annually by the Chief Forensic Toxicologist and updated as needed to reflect any changes in policies or procedures. The manual governs operations in the Little Rock Forensic Toxicology laboratory.

It is recognized that unforeseen circumstances may arise which require immediate deviations from the policies and procedures of this manual. If this deviation affects multiple cases, the request for an exception to policy will be submitted to the Chief Forensic Toxicologist, Technical Leader, 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.

New policies may be approved and distributed by the Section Chief, as may interpretations, clarifications, or expansions of existing policies. Changes to any manual require a revision of the affected document through the Qualtrax system. Interpretations and clarifications of existing policy will be distributed in writing to all affected.

5.6 QUALITY MANAGEMENT

See *ASCL-DOC-01 Quality Manual*.

5.7 MANAGEMENT SYSTEM COMMUNICATION AND INTEGRITY

ASCL methods of communication include regular Section Chief meetings, Discipline meetings, labwide meetings, email, telephone, and personal meetings.

ASCL management communicates the importance of meeting customer, statutory, and regulatory requirements during regular Section Chief and labwide meetings. Pertinent items from the Section Chief meeting minutes may be distributed by email, when appropriate.

All communication with parties outside of the laboratory must be in compliance with A.C.A. §12-12-312 and laboratory policy. Work-related emails to these external parties may be copied (by CC or BCC) to the Section Chief.

6 RESOURCE REQUIREMENTS

6.1 GENERAL

The laboratory shall have available the personnel, facilities, equipment, systems and support services necessary to manage and perform its laboratory activities.

6.2 PERSONNEL

6.2.1 PERSONNEL

The Forensic Toxicology Section complies with the lab wide policy regarding personnel matters. All staff who could influence the activities of the laboratory will act impartially, be competent, and work in accordance with the ASCL management system.

6.2.2 COMPETENCE REQUIREMENTS

See *ASCL-DOC-01 Quality Manual*.

6.2.2.1 EDUCATION

Forensic Toxicologists who authorize results, opinions and/or interpretations shall possess a baccalaureate degree in chemical, physical, biological science, or forensic science.

6.2.2.2 TRAINING PROGRAM

Each Forensic Toxicologist, regardless of prior training or experience, must complete a training program prior to assuming casework responsibilities. For analysts with prior experience, this training may be truncated with the approval of the Chief Forensic Toxicologist and the Assistant Director, or designee. A (possibly) truncated version of this training program can also serve as the basis for remedial or refresher training of existing employees.

The Chief Forensic Toxicologist ensures the competence of all personnel who operate specific equipment, perform analyses, evaluate, review or verify results, or issue reports of laboratory analysis in this category of testing. This is accomplished by:

- Requiring the successful completion of a baccalaureate degree program in chemistry, biology, or other natural science or closely-related field
- Requiring the successful completion of the Forensic Toxicology Section training program, including a competency test and moot court
- Verifying ongoing compliance through full technical and administrative review of casework

The training program is detailed in the *Forensic Toxicology Section Training Manual* (*TOX-DOC-002*). Among the contents of this training are:

- The knowledge, skills, and abilities needed to perform work
- General knowledge of forensic science
- The application of ethical practices in forensic science
- Criminal/civil law procedures and testimony
- Provisions for retraining
- Provisions for maintenance of skills and expertise
- Criteria for acceptable performance
- Health and safety requirements
- Laboratory policies and procedures
- Instrumentation theory and practice
- Evidence handling and sampling procedures
- Analytical techniques and instrumentation
- Moot court
- Quality system requirements
- Interpretation and reporting
- Competency testing

Records will be maintained which document what training has occurred, and the evaluation(s) of that training.

Written tests will occur to document the trainee's knowledge of the subject material. Oral examinations may also be utilized to demonstrate the trainee's knowledge.

Training on new procedures will be documented for existing employees as the new procedure(s) are brought online. Training will include observation of the method and, if appropriate, a successful competency test. Both observation and completion of a proficiency test will need to be documented in the employees training binder or appropriate place.

6.2.3 COMPETENCE OF STAFF

The ASCL ensures the competence of all personnel to perform the tasks for which they are responsible, and to evaluate the significance of any deviations from policy and/or procedure.

6.2.3.1 COMPETENCY TESTING

The competency specimens in the Forensic Toxicology Section are intended to mimic typically encountered specimens. They encompass a range of specimen types and analyte classes. It is not necessary to include every test method in the competency test, but commonly-performed test methods will be represented.

A written report will be generated and evaluated as though it were a normal case. The intended result(s) of the competency test shall be achieved and documented prior to performing the covered task(s) on actual items of evidence. This may be achieved in several ways, including:

- Observed testing on a surrogate item, such as old proficiency test material
- Written examination
- Oral examination

The risk involved will be considered when determining the extent of the competency test.

For laboratory personnel whose job responsibility includes report writing, a competency test shall include, at a minimum:

- Practical examination of sufficient unknown samples to cover the anticipated spectrum of assigned testing tasks, to evaluate the individual's ability to properly perform analysis
- A written report to demonstrate the individual's ability to properly convey results and/or conclusions and the significance of those results and/or conclusions.
- A written or oral examination to access the individuals knowledge of the discipline, category of testing, or task being performed, and
- Moot court to demonstrate the individual's ability to properly convey and present results of evidence in court.

6.2.3.2 COMPETENCY–TESTED ACTIVITIES

Competency testing for the following activities will be conducted and documented prior to these actions being performed on evidence:

- Laboratory activities (testing and/or sampling)
- Analysis of results
- Review of results
- Authorization of results
- Technical review
- Expressing an opinion or interpretation

6.2.4 DUTIES, RESPONSIBILITIES, AND AUTHORITIES

The duties, responsibilities, and authorities of each position are contained in its job description which can be found in §5.2.7, above in this quality manual.

6.2.5 PERSONNEL REQUIREMENTS

The Forensic Toxicology Section complies with the lab-wide policy regarding personnel requirements.

The Chief Forensic Toxicologist ensures the competence of all personnel who operate specific equipment, perform analyses, evaluate, review or verify results, or issue reports of laboratory analysis in this category of testing. This is accomplished by:

- Requiring the successful completion of a baccalaureate degree program in chemical, physical, or biological science, or forensic science.
- Requiring the successful completion of the Forensic Toxicology Section training program, including a competency test and moot court
- Verifying ongoing compliance through full technical and administrative review of casework

6.2.6 AUTHORIZATIONS

The Section Chief authorizes personnel to perform certain duties. Personnel may not perform these duties without authorization, except during supervised training. These duties include:

- Performing testing activities (e.g., testing/examination, sampling, reagent preparation)
  - Use of equipment (as applicable)[1]
- Method development, modification, verification, and/or validation of methods
- Analysis of results, including:
  - Statements of conformity
  - Opinions/interpretations
- Reporting results
- Reviewing Results
- Authorizing results

Authorization is documented on the *Analyst and Technician Competency Authorization Documentation* form (ASCL-FORM-62) and maintained in the Personnel tab of Qualtrax. Qualtrax shall also contain a curriculum vitæ or résumé that includes educational and professional qualifications, training, skills, and experience. The individual's Training Binder will contain all completed training records.

6.3 FACILITIES AND ENVIRONMENTAL CONDITIONS

6.3.1 GENERAL

See *ASCL-DOC-01 Quality Manual*.

6.3.2 DOCUMENTATION

If environmental conditions are such that the validity or reliability of analytical results could be jeopardized, testing will be stopped until those conditions can be remediated.

[1] Trainees are authorized to use equipment as required for the training process.

6.3.3 MONITORING RECORDS

A record of the temperature conditions for all evidence storage locations within the section will be maintained. Refrigerated storage for evidence should be kept between -1°C and 4°C. Refrigerated storage for chemicals should be kept between 2°C and 8°C. Frozen storage should be kept at or below 0°C. If the storage conditions deviate from that range for an extended time period (i.e., more than one day) then the cause will be assessed and any necessary action taken.

6.3.4 CONTROL OF FACILITIES

The manual governs operations in the Little Rock Forensic Toxicology laboratory.

If environmental conditions are such that the validity or reliability of analytical results could be jeopardized, testing will be stopped until those conditions can be remediated.

6.3.4.1 ACCESS

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 magnetically-locked doors which prevent access from the central hallways without a security access card. Because the lab area is secured at all times in this manner, the doors inside the Forensic Toxicology section need not be locked either during working hours or after working hours. Section refrigerated specimen storage is a common storage area which is locked when not in use or under direct observation.

Any controlled substances (in powder form) present in the section are kept in a locked drawer in the laboratory area (room 327). Only Forensic Toxicologists and Toxicology Technicians have a key to this drawer. Controlled substances in solution do not require secure storage conditions.

6.3.4.1.1 KEY BOX

The Chief Forensic Toxicologist maintains the section key box containing cabinet keys. Keys are distributed to authorized personnel only, and a log is kept of all key transfers. The section key box is inventoried annually.

6.3.4.2 PREVENTION OF ADVERSE INFLUENCES

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
- Solvent-rinsing of any cleaned, reused glassware
- Use of 1:1 checks of all transfers between labelled containers

6.3.4.3 SEPARATION

The Forensic Toxicology Section is located adjacent to the Forensic Chemistry Section but has effective separation from it. The two sections are separated by a door which is closed when not in use.

6.3.5 EXTERNAL ACTIVITIES

See *ASCL-DOC-01 Quality Manual*.

6.4 EQUIPMENT

6.4.1 ACCESS

Only individuals who have been trained in the proper use of the instrumentation/equipment are authorized to use it.

6.4.2 OUTSIDE EQUIPMENT

If the ASCL must use equipment outside of its permanent control, the laboratory shall ensure that the equipment meets the requirements of this section.

Successful performance verification is required for any equipment that has gone outside of the direct control of the laboratory (e.g., pipettes shipped to an external provider for repair or preventive maintenance) before that equipment may be returned to service. Documentation of these verifications will be maintained on the Toxicology shared drive.

6.4.3 PROPER FUNCTIONING

The equipment used in the Forensic Toxicology section is as follows: Randox, UV-Vis, Indiko, Gas Chromatograph, Liquid Chromatograph, Mass Spectrometry, balances, micropipettes, reference standards, certified reference materials (CRM), reagents, glassware (calibrated volumetric measuring devices), pipettes (calibrated volumetric measuring devices) and solvents. All purchased chemicals, reference materials/standards, and disposable equipment are considered fit for use when received.

All equipment will be maintained in a clean, orderly, and safe condition. Procedures for equipment to ensure proper functioning and prevent contamination or deterioration include:

- Handling
- Transport
- Storage
- Use
- Planned maintenance

HANDLING

Laboratory equipment and instrumentation shall be handled responsibly to ensure optimal performance and to avoid contamination and premature wear/damage.

TRANSPORT

Laboratory equipment and instrumentation shall be transported responsibly to ensure optimal performance and to avoid contamination and premature wear/damage.

If reference materials, such as CRMs are transported, care will be taken to ensure that their storage conditions (e.g., temperature control) are appropriate during transport.

Successful performance verification is required for specific equipment (e.g., pipettes) that has been transported from one ASCL laboratory location to another before that equipment may be returned to service. Reference materials transported between locations do not require additional performance verification. Documentation of these verifications will be maintained on the Toxicology shared drive.

STORAGE

Reference materials, such as CRMs, will be stored in the manner listed by their manufacturer, or another similar manner intended to protect the material from deleterious change, when practicable.

USE

Only individuals who have been trained in the proper use of the equipment shall operate it. Proper use of equipment will be covered within specific testing methods.

PLANNED 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.

If new equipment requires a validation, the personnel must be trained before they can use the instrument in casework. This training will be documented in Qualtrax.

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:

- Record of all calibration and quality control checks
- Record of all maintenance performed on the instrument

An outline of normal operating parameters (e.g., oven program, gas flow rate) will be kept on the shared Toxicology drive.

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

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 or appropriate LIMS case file. 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 toxicologist will assess the calibration by examining the autotune report for the following items:

- 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
- If the EM voltage is greater than 2500

If either of these conditions exists, the instrument is not in proper working condition and will 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 in the appropriate LIMS case file.

If the tune report indicates that the tune is acceptable, the analyst checking the tune report will index it into the appropriate LIMS folder.

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:

- The source should be cleaned following manufacturer-recommended procedures
- The filaments should be replaced
- The diffusion pump oil should be inspected and replaced if necessary
- The fore-line pump oil should be checked and filled or replaced if necessary
- The vent line should be rinsed with methanol
- The vent line trap should be inspected and replaced if necessary
- The gold seal should be inspected and replaced if necessary
- The inlet should be scrubbed with a wire brush and/or methanol to remove minor debris

LIQUID CHROMATOGRAPH-MASS SPECTROMETERS

The guard column filter should be changed routinely, typically after approximately one-hundred injections of a biological extract.

The AB Sciex LC-MS will be tuned when necessary, typically during a preventive maintenance visit or when the tune drifts more than 0.4 Daltons (as judged by the components of a positive control mix).

The Agilent LC-MSs will be check-tuned at least weekly, if used, and should be tuned before each sequence is run.

UV-VISIBLE SPECTROPHOTOMETER

UV-Vis uses positive control samples supplied by IL Instrumentation Laboratory (or equivalent), 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 positive control and a negative control (consisting of blank blood) are run with each batch of casework.

INDIKO PLUS

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

RANDOX EVIDENCE INVESTIGATOR OR EVIDENCE+

The Randox Evidence Investigator and Randox Evidence+ are maintained according to the manufacturer's specifications. Maintenance is performed as necessary (if used). Camera calibration should be performed once a month on the Randox Evidence Investigator. Control samples are run daily (if 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 adjusted. The acceptability range for balances can be found in § 6.4.7. The procedure for adjustment will vary from balance to balance.

PIPETTES

Micropipette calibration will be checked each calendar year, and the micropipettes recalibrated and/or repaired, if necessary. Calibration services are provided by an ISO/IEC 17025 accredited vendor.

The acceptability criterion for the "as found" value of micropipettes is 8% or less for single-channel micropipettes and 16% or less for multichannel pipettes. Micropipettes should be evaluated every six months, including the annual outside vendor calibration. A record of semiannual pipette checks will be kept on the section shared drive.

Pipettes which do not contribute to uncertainty of measurement (e.g., pipette for carboxyhemoglobin testing, multichannel pipettes) do not require semiannual pipette checks. If a pipette is found to be outside of the acceptability criteria, the pipette will be removed from service until it can be calibrated or repaired and a corrective action will be started.

REFERENCE STANDARDS

The Forensic Toxicology Section maintains a 5 gram NIST-certified reference mass standard and a 100 gram NIST-certified reference mass standard for use in performance adjustments to its balances. The certified reference mass shall be handled responsibly to prevent contamination or deterioration and to protect its integrity. It is the supervisor or designee's responsibility to ensure that proper planning and care is taken.

Micropipettes are calibrated at least once per calendar year by an authorized external calibration service provider.

Micropipettes and balances used for critical measurements (i.e., measurements which can have a significant effect on a reported result) are specifically identified in the case record, typically through the use of a batch worksheet and/or results worksheet.

NIST certified weights are used to conduct performance verifications and adjust the balances used for casework. The weights will be calibrated or replaced every ten years. The calibration or validation records will be retained by the section.

REFERENCE DATABASES

Mass spectral and other libraries used to identify unknown compounds are well accepted in the field and will be uniquely identified.

6.4.3.1 REAGENT RECORDS AND LABELING

The Forensic Toxicology Section complies with the lab-wide policy regarding reagents, chemicals, and standards. General safe-handling guidelines may be found in the *Health and Safety Manual* (ASCL-DOC-08).

Reagents, chemicals, and standards are of known quality and are subject to quality control requirements to ensure that they are fit for use.

Except where otherwise noted, purchased chemicals should be of ACS Reagent grade or better. All purchased solvents, chemical, reagents, reference materials shall be marked when received with the date and initials of the person receiving them. Upon opening, the bottles shall be marked with the date and initials of the individual opening the substance.

Water used for aqueous preparations should be deionized whenever possible.

Reference materials used for controls must be verified to ensure that they are fit for use. Acceptable methods of verification include a certificate of analysis, characterization by mass spectrometry (to detect the compound and any breakdown products), comparison to a known standard by gas chromatography, or similar.

Records of all verifications will be kept in the Toxicology shared drive or maintained in Qualtrax. Reference materials, such as CRMs, will be stored in the manner listed by their manufacturer, or another similar manner intended to protect the material from deleterious change, when practicable. If they are transported, care will be taken to ensure that their storage conditions are appropriate during transport. All controls will be logged in a logbook with the following information:

- Source
- Lot number, if available
- Date received/prepared
- Unique identifier
- Expiration date, if appropriate
- Verification results, if appropriate

When a new reference material is received, the following procedure is followed:

1) Mark the container with the date received and the initials of the person who received it.
2) Scan the appropriate certificate of analysis into the appropriate folder on the Tox shared drive with the date that sample was received.
3) Ensure that the reference material is verified before analytical results based on it are released. A certificate of analysis suffices for verification. If no Certificate of Analysis is available then an unextracted sample of the reference material may be analyzed using mass spectrometry to evaluate whether the composition of the reference material is consistent with the stated purity.

When a chemical or reagent is prepared, its fitness for use must be demonstrated. This can be achieved by use of an assay if:

- It is run for comparison with the same chemical or reagent which was previously verified, or
- Positive and negative controls are analyzed in the assay and respond appropriately.

When a new reference material solution is prepared, the following procedure is followed:

1) Add an entry to the Standard Preparation Log (TOX-FORM-006) listing the drug name(s), vendor(s), preparer, lot number(s), preparation date, the final concentration(s) of the reference material, and an expiration date.
2) A standard number is assigned to the reference material. This number is generally assigned sequentially or may be set using a specific year (e.g., for 2020 begin with the number set 2001).
    a) The standard number is prepended with an alphabetical code to give information about the standard.
        i) CE for standards purchased from Cerilliant
        ii) GR for standards purchased from Grace
        iii) ET for ethanol standards
        iv) TM for test mixes
        v) MX for other mixed standards
        vi) Other abbreviations, as needed
    b) The standard number is appended with an alphabetical code to indicate the concentration of the component(s)
        i) A for a 1 mg/mL solution, and increment the letter by one for each 1:10 dilution
        ii) In a mixture, the highest concentration determines the suffix
        iii) X,Y, and Z are reserved for cases where the appropriate suffix is unclear
        iv) Subsequent preparations of dilutions from the same stock will append an incremented number (e.g., the second preparation of CE123C would be labeled CE123C2, and entered separately into the Standard Preparation Log)
3) If the reference material is used to make a calibration curve, or as a positive control in the initial verification of a calibration curve, the result(s) of this analysis are evaluated. Any reference materials which are found to be unsuitable for quantitative use will be discarded, or clearly labeled as for qualitative use only. Calibrated volumetric measuring devices[2] must be used

[2] (Micropipettors, class A volumetric flasks)

when preparing certified reference materials for quantitative analysis (e.g., calibrators, controls). Calibrated equipment does not need to be used when preparing non critical reagents and solutions (e.g., internal standard solutions, buffers, mobile phase, Base Test Mix)

6.4.3.2 REFERENCE COLLECTION RECORDS

The Forensic Toxicology Section uses reference collections for comparison to known reference materials in the GC-MS testing technique. Each reference collection has entries documented, uniquely identified, and properly protected.

6.4.4 PERFORMANCE VERIFICATION

Before a new instrument is first placed into service, performance verification will be performed to ensure that the instrument is fit for use with the appropriate method. Positive and negative controls and/or calibrators are sufficient for this purpose. The documentation (or a reference to its location) will be maintained in the maintenance log for that instrument.

6.4.5 FITNESS FOR SERVICE

All equipment used for measurement will be capable of achieving the measurement accuracy and/or measurement uncertainty required to provide a valid result.

6.4.6 CALIBRATION REQUIREMENT

Measuring equipment will be calibrated when:

- The measurement accuracy or measurement uncertainty affects the validity of the reported results, and/or
- Calibration is required to establish metrological traceability of the reported results (i.e., to SI units)

6.4.7 CALIBRATION PROGRAM

The Forensic Toxicology Section complies with the lab-wide policy regarding a calibration program which is reviewed and adjusted as necessary to maintain confidence in the status of calibration.

Listed below is the equipment with its calibration interval. The equipment will be calibrated by a service provider accredited to ISO/IEC 17025 accredited calibration laboratory or replaced after the calibration interval has passed.

Calibration certificates shall contain the measurement results, including the measurement uncertainty or a statement of compliance with an identified metrological specification.

Equipment	Calibration Interval	Tolerance
Balances, Analytical	5 years	±0.0005 g
Balances, Toploading	5 years	±0.1 g
Traceable weights (performance checks)	10 years	0.5 mg
Certified reference materials	See CoA	See CoA
Glassware	5 years	Varies
Micropipettes	1 year	See §6.4.3

6.4.7.1 COMPONENTS

Equipment requiring calibration is listed below. The specific requirements for calibration and interval can be found in §6.4.7 of the *Forensic Toxicology Quality Manual* (*TOX-DOC-01 Quality Manual*)

- Balances
- Micropipettes
- Glassware (e.g., volumetric flasks, serological pipettes)
- 5 gram or 100 gram NIST-certified reference mass
- Reference materials (i.e., certified reference materials)

The Forensic Toxicology Section purchases certified reference materials from companies which provide a certificate of analysis. These certificates of analysis are maintained on the shared Toxicology network drive or in Qualtrax. Materials for which no certificate of analysis is provided must be verified before use. Reference materials for which the expiration date has passed must be re-verified before use (e.g., through comparison to a calibration curve, mass spectral analysis).

Thermometers for the Forensic Toxicology section shall be NIST-Traceable or equivalent and are not subject to calibration. Thermometers should be performance checked annually or replaced as necessary.

Certified reference materials used in critical measurements (i.e., measurements which can have a significant effect on a reported result) are specifically identified in the case record, typically through the use of a batch worksheet and/or results worksheet.

For qualitative analysis only, where certified reference materials are not readily available, the manufacturer's listed contents may suffice for this verification.

6.4.8 LABELLING

All equipment[3] that is calibrated, or has a defined period of validity, will be marked so as to indicate either the calibration status or the period of validity.

[3] Including reagents

6.4.9 OUT OF SERVICE

Any equipment which has been subjected to overloading or mishandling, gives questionable results, or has been shown to be defective or outside specified requirements, shall be taken out of service.

It will be labeled as "Out of Service" or isolated from functional equipment to prevent its use. It will only be returned to service after it has been verified to perform correctly.

When equipment is removed from service due to misuse, a *Quality Assurance Concern* workflow is initiated in Qualtrax, and the ASCL will examine any effect that the deviation may have had on its activities.

6.4.10 INTERMEDIATE CHECKS

Micropipettes should be evaluated every six months including outside vendor calibration annually. This procedure is listed in § 6.4.3 of this manual.

Balances are subjected to intermediate checks. This procedure is listed in § 6.4.3 of this manual.

6.4.11 CORRECTIVE FACTORS

See *ASCL-DOC-01 Quality Manual*.

6.4.12 EQUIPMENT ADJUSTMENT

If unintended adjustments of equipment may influence testing results, the discipline will take precautions (when practicable) to prevent these unintended adjustments. This may be accomplished by, for example:

- Using positive and negative controls, standards, or known reference material at the beginning and end of instrumental runs/analytical sequences
- Placing tamper-proof seals over the adjustment points
- Specifying dedicated personnel as the only individual(s) authorized to make the adjustments

6.4.13 EQUIPMENT RECORDS

Records are retained for equipment that influences laboratory activities. These records include the Forensic Toxicology Equipment Log, Calibration Certificates, Reagent Logbooks, Instrument Logs, Balance Logs, and Pipette Logs.

Information to be retained in these records includes:

a) the identity of equipment, including software and firmware version;
b) the manufacturer's name, type identification, and serial number or other unique identification;
c) evidence of verification that equipment conforms with specified requirements;
d) the current location;

e) calibration dates, results of calibrations, adjustments, acceptance criteria, and the due date of the next calibration or the calibration interval;
f) documentation of reference materials, results, acceptance criteria, relevant dates and the period of validity;
g) the maintenance plan and maintenance carried out to date, where relevant to the performance of the equipment;
h) details of any damage, malfunction, modification to, or repair of, the equipment;
i) the LIMS instrument case number(s) if applicable;
j) the identifier used to identify the instrument in case work, if applicable;
k) the date the equipment was permanently retired from service, if applicable

When equipment is retired, the records shall be maintained and available for at least one full accreditation cycle.

6.5 METROLOGICAL TRACEABILITY

6.5.1 GENERAL

See *ASCL-DOC-01 Quality Manual*.

6.5.1.1 SUPPLIER REQUIREMENTS

If a material or service must meet certain specifications in order to properly function in testing, these items and the required specification(s) will be communicated to the Procurement Section, generally through Qualtrax.

Supplies, reagents, and consumable materials that affect the quality of tests are not used until they have been verified to meet the previously-defined specifications. Inconsistencies will be reconciled before materials are utilized in casework.

As chemicals are first opened in the section, the opener is responsible for initialing and dating the container. Supplies, reagents, and consumable materials shall be stored in accordance with the manufacturer's recommendations.

Critical consumables, supplies, and services which affect the quality of testing will be obtained from reliable suppliers.

In the Forensic Toxicology Section, the critical consumables are:

- Certified standards/reference materials
- Immunoassay kits
- Drug quantitation kits (Tox Boxes)
- PFTBA (perfluorotributylamine) GC-MS tuning compound

In the Forensic Toxicology Section, the critical supplies are:

- Certified reference mass (for balance adjustment)

6.5.1.2 ALTERNATE SUPPLIER REQUIREMENTS

See *ASCL-DOC-01 Quality Manual*.

6.5.1.3 INTERNAL CALIBRATION

See *ASCL-DOC-01 Quality Manual*.

6.5.1.4 CRM ALTERATION

If a certified reference material is changed in a way that alters its traceable measurement value[4], then the equipment used to alter the material will be evaluated for applicability of measurement traceability requirements[5].

6.5.2 TRACEABILITY TO THE INTERNATIONAL SYSTEM OF UNITS

The Forensic Toxicology Section maintains a 5 gram NIST-certified reference mass standard and a 100 gram NIST-certified reference mass standard for use in performance adjustments to its balances. The certified reference mass shall be handled responsibly to prevent contamination or deterioration and to protect its integrity. It is the supervisor or designee's responsibility to ensure that proper planning and care is taken.

NIST certified weights are used to conduct performance verifications and adjust the balances used for casework. The weights will be calibrated or replaced every ten years. The calibration or validation records will be retained by the section.

Thermometers for the Forensic Toxicology section shall be NIST-Traceable or equivalent and are not subject to calibration. Thermometers should be performance checked annually or replaced as necessary.

Batch worksheets are used to document the traceability of certified reference materials and measurement equipment. Micropipettes used for measurements which can have a significant effect on a reported result will be identified on the batch worksheet or the results worksheet. Certified reference materials used in an assay will be identified on the batch worksheet or the results worksheet.

Batch worksheets are also used to record the evaluation of control results, if those controls are not otherwise contained in the case file. This evaluation is performed by a second qualified analyst.

[4] For example, the dilution of a solution with a certified concentration of an analyte
[5] For example, the metrological traceability of a calibrated volumetric measuring device

Batch worksheets, and the control data associated with them, are stored in the LIMS in case file dedicated to this purpose.

The Forensic Toxicology Section purchases certified reference materials from companies which provide a certificate of analysis. These certificates of analysis are maintained in Qualtrax. Materials for which no certificate of analysis is provided must be verified before use. Reference materials for which the expiration date has passed must be re-verified before use (e.g., through comparison to a calibration curve, mass spectral analysis, infrared analysis).

Certified reference materials used in critical measurements (i.e., measurements which can have a significant effect on a reported result) are specifically identified in the case record, typically through the use of a batch worksheet and/or results worksheet.

Reference standards and materials shall be handled responsibly to prevent contamination or deterioration and to protect their integrity. It is the supervisor or designee's responsibility to ensure that proper planning and care is taken.

6.5.3 ALTERNATE TRACEABILITY

For qualitative analysis only, where certified reference materials are not readily available, the manufacturer's listed contents may suffice for this verification.

6.6 EXTERNALLY-PROVIDED PRODUCTS AND SERVICES

6.6.1 GENERAL

Only suitable externally-provided products and services will be used, when they are:

a) Intended for incorporation of the lab's own activities[6], or
b) Provided directly to the customer as received by the laboratory[7], or
c) Used to support the operation of the laboratory[8]

6.6.2 RECORDS

If the Arkansas State Crime Laboratory transfers evidence to an outside laboratory[9], an *Inter-Laboratory Evidence Transfer Form* (ASCL-FORM-07) must be completed and entered into the case file. The Inter-Laboratory Evidence Form may be waived for items funded out of a grant and/or items under a contract. Any cost incurred by the laboratory must be approved by the Fiscal Officer, or designee.

[6] For example: outsourced analysis used by an ASCL analyst as the basis of an opinion/interpretation
[7] For example: outsourced analytical report forwarded to a customer without modification
[8] For example: consumable materials, reference materials, equipment maintenance, proficiency testing services, calibration services
[9] For example: NMS Laboratories or Axis Forensic Toxicology Laboratory

All external laboratories performing casework for the Arkansas State Crime Laboratory must be accredited by an accrediting body recognized by the Arkansas State Crime Laboratory. These laboratories must provide the Arkansas State Crime Laboratory with documentation of accreditation, which is maintained in Qualtrax.

6.6.3 COMMUNICATION

The ASCL will communicate its requirements (if any) to external providers for:

a) the products and services to be provided
b) the acceptance criteria
c) competence, including any required qualification of personnel
d) activities that the laboratory, or its customer, intends to perform at the external provider's premises

7 PROCESS REQUIREMENTS

7.1 REVIEW OF REQUESTS, TENDERS, AND CONTRACTS

7.1.1 GENERAL

The Forensic Toxicology Section processes evidence submitted by external law enforcement agencies and the Medical Examiner's Office of the Arkansas State Crime Laboratory. Contracts (submission sheets) are reviewed by the Forensic Toxicology personnel to assess the requests made by the customer; if any changes or amendments are necessary, all affected personnel shall be notified.

Once accepted by the laboratory, the laboratory agrees to test submitted evidence in accordance with laboratory policies and procedures as described in this manual.

By completing and submitting the submission sheet, each customer relinquishes all decisions regarding analytical processing and the choice of methods to the laboratory.

Any testing for which there is not a validated method must be approved in writing by the Chief Forensic Toxicologist, by placing their initials and the date by on the ASCL Evidence Submission Form next to the request.

Before analysis begins, the analyst reviews the request to determine what testing is appropriate. There is no requirement to perform the specific testing requested by the customer on the *ASCL Evidence Submission Form* (ASCL-FORM-12), but the request (and its purpose, if known) guides the decision as to what testing is appropriate.

The Medical Examiner Section is considered an internal customer, and the review of their requests, tenders, and contracts may be performed in a simplified manner. The *Medical Examiner/Forensic Toxicology Section Submission Form* (TOX-FORM-01) contains a detailed list of analysis types, and a cursory review of the requested testing will be made by the analyst when deciding the course of analysis. No record of this review is necessary.

The actual testing performed for the Medical Examiner Section may differ from the analysis requested on their submission form. If this deviation is routine (e.g., not testing multiple specimen types for volatiles if the blood is negative), then such changes do not require notification of the requesting pathologist. Other, more substantive changes to the requested testing may require notification of the requesting pathologist (or the Chief Medical Examiner if the requesting pathologist is not available).

The course of analysis determined by an evaluation of the purpose of the submission, the facts of the case, and the constraints imposed by external factors (e.g., specimen amount, method limitations).

The goal of the Forensic Toxicology Section is to answer the purpose of the submission using the most appropriate assay(s), the least specimen, and the most efficiency.

If the specimen amount is insufficient to perform all of the analyses requested, the analyst attempts 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 acceptable to analyze a smaller-than-normal sample amount if necessary, but a disclaimer accompanies any negative findings indicating that insufficient sample was available for normal testing. This disclaimer is not required if at least one normal aliquot of the specimen has been analyzed for each type of analysis reported.

Sample containers containing a preservative (e.g., a gray-stoppered tube) should be used whenever possible and appropriate. Green-stoppered tubes may not be appropriate volatiles analysis due to potential interferences (e.g., toluene positives) with data interpretation from the preservative (heparin). If toluene is detected in a specimen with a green stoppered tube it will not be reported as present.

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

The review of the customer's request, as stated above, will also cover any work which is subcontracted to another laboratory.

The Forensic Toxicology Section complies with the lab-wide policy regarding subcontracting. The Forensic Toxicology Section will occasionally subcontract testing to an outside laboratory. Testing must be performed by a qualified and approved laboratory. A register of approved subcontractors is maintained by the Quality Assurance Manager.

Cases undergo testing 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., NAME accreditation)
- Priority/rush requests
- Convenience of analysis (e.g., analyzing samples in batches for reasons of economy of scale)

Stat priority cases are assigned to any available analyst.

INITIAL SCREENING

An initial screening is routinely performed on cases after accession. This screening normally consists of a blood alcohol or immunoassay test (as appropriate).

IMPAIRMENT CASES

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

1) Initial screening
   a) Blood alcohol analysis (if appropriate)
   b) Immunoassay screening (if appropriate)
2) LC-MS or GC-MS analysis (if appropriate)
3) THC confirmation (if appropriate)
4) 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. At or above these values, the subject is considered legally intoxicated, and further analysis is unnecessary to demonstrate intoxication unless a DRE evaluation has been performed.

Driver	Per se limit (g% ethanol)	Statute
Under 21 years-of-age	0.02	A.C.A §5-65-303
Commercial drivers or aircraft operators	0.04	A.C.A §27-23-114
Other drivers	0.08	A.C.A §5-65-203

Urine alcohol quantitative results will be reported unless it is indicated that the urine was not collected according to guidelines promulgated by the Arkansas Department of Health Office of Alcohol Testing (i.e., an initial voiding of the bladder, a thirty minute waiting period, and a second voiding of the bladder for urine collection). When urine samples are not collected properly, urine volatiles testing may be performed and reported qualitatively. Drug-facilitated sexual assault cases where urine is submitted for ethanol testing will be reported quantitatively.

If urine is available, a urine immunoassay screen may be performed. If only blood is available, an immunoassay or further screening 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 confirmation is performed, as appropriate. 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.

Additional testing may be required in some cases. The course of additional testing depends on the details of the case. Fatality motor vehicle accidents with a positive immunoassay require qualitative drug testing.

OTHER CASES

The course of analysis for other law enforcement cases is dependent upon the needs of each individual case. A course of analysis is 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 normally tested.

MEDICAL EXAMINER CASES

The progression of analysis for Medical Examiner and Coroner cases is as follows:

1) Stat alcohol and/or COHb analysis (if requested)
2) Volatiles analysis
3) Initial screening
    a) Immunoassay screening (if requested) blood alcohol (if requested)
    b) COHb analysis (if requested)
4) Blood drug screening (acid and base extractions, if requested)
5) GC-MS or LC-MS drug screening/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 are run on the GC-MS. Efforts should be made to keep one milliliter of specimen in reserve, if possible, for further testing.

Other testing may be required based on the needs of each case. When a specific drug is requested for identification or quantitation by the pathologist, this should be addressed in the case file and the final report. An extracted-ion chromatogram will show the presence or absence of the drug in question.

DISPOSAL

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

- 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.
- 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 may be destroyed after one year if no request for retention or further testing has been received. The chain of custody serves as a record of all specimens destroyed. It lists 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.

Evidence for a particular case may also be transferred to the submitting agency for long term storage or via a court order to a lab of an attorneys choosing. Samples will not routinely be retained beyond the retention policy of six months for non-homicide cases or one year for homicide cases.

RELEASE OF EVIDENCE ITEMS

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.

CESSATION OF ANALYSIS

The Forensic Toxicology Section may discontinue further forensic examinations when the toxicological results support the maximum charge to be filed, or if further testing is otherwise inappropriate.

7.1.2 INAPPROPRIATE REQUESTS

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 to 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 of forcible sexual assault
- 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. Beverages will not be analyzed for ethanol presence and possible content
- Cases where serum only is submitted for testing
- Cases where oral fluid only is submitted for testing

Exceptions may be made to this policy when testing may be appropriate. The Forensic Toxicology Section may discontinue further forensic examinations when the toxicological results support the maximum charge to be filed, or if further testing is otherwise inappropriate.

7.1.3 STATEMENTS OF CONFORMITY

The Forensic Toxicology Section does not issue reports containing statements of conformity.

7.1.4 RESOLUTION OF DIFFERENCES

Any difference between the request or tender and the contract shall be resolved before any work commences. Each contract shall be acceptable both to the ASCL and the customer.

7.1.5 DEVIATION FROM THE CONTRACT

Although the laboratory is responsible for determining what testing is appropriate and necessary, the customer will be notified if a request for analysis is canceled altogether. This notification may be by telephone, electronic mail, facsimile, a request status of 'canceled' on iResults, or the equivalent.

7.1.6 AMENDMENT OF THE CONTRACT

All affected personnel will be notified if the contract needs to be amended after work has begun.

7.1.7 COOPERATION WITH CUSTOMERS

See *ASCL-DOC-01 Quality Manual*.

7.1.8 RECORDS OF REVIEW

See *ASCL-DOC-01 Quality Manual*.

7.1.9 DATABASE SEARCH EXTENT

See *ASCL-DOC-01 Quality Manual*.

7.2 SELECTION, VERIFICATION, AND VALIDATION OF METHODS

7.2.1 SELECTION AND VERIFICATION OF METHODS

See *ASCL-DOC-01 Quality Manual*. Forensic Toxicology's test methods are listed in §9 of this manual.

For §7.2.1.1-7.2.1.6 See *ASCL-DOC-01 Quality Manual*.

7.2.1.7 DEVIATION FROM METHOD

Standard analytical procedures are important in ensuring quality. Standardized test methods 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 these test methods must be documented in the case file. The Chief Forensic Toxicologist will keep a log of method/procedure deviations.

7.2.2 VALIDATION OF METHODS

7.2.2.1 EXTENT OF VALIDATION

Prior to implementing a non-standard method, a laboratory-developed method, a standard method used outside its intended scope, or amplifications and modifications of a standard method, a validation will be performed. The validation will be as extensive as necessary to ensure that the method if fit for service and meets the needs of the given application.

The techniques used for validation can include one or more of:

- Evaluation of bias and precision using reference standards or material
- A systemic assessment of the factors influencing the result
- Testing method robustness by varying controlled parameters
- Comparison of results achieved with other validated methods
- Inter-laboratory comparisons
- Evaluation of the measurement uncertainty of the results, based on an understanding of the theoretical principles of the method, and practical experience of the performance of the method

7.2.2.1.1 VALIDATION PROCEDURE

Before the validation begins, a validation plan will be approved by the Section Chief, Quality Assurance Manager, and the Technical Leader (if appropriate). The validation plan should address the expected performance of the method as it relates to the needs of the customer. The validation plan shall be updated as necessary and affected personnel will be notified.

Once the validation plan is approved, the validation may begin. Elements included in the validation will include:

- Associated data analysis/interpretation
- The data required to report a result, opinion, or interpretation
- Any limitations of the method, including reported results, opinions, or interpretations

Whenever practicable, validation shall also involve the use of at least one of the following procedures:

- Split samples
- Blind trials
- Concordance testing

Validation guidelines promulgated by reputable technical organizations (e.g., SWG-TOX) may alternately be used to determine the structure and extent of the validation process.

Following approval of the validation, individuals will be trained by the personnel involved in performing the validation. This training will include the interpretation of results, quality assurance and quality control measures, and documentation requirements. The training will be performed prior to use of the new analytical procedure in casework and must be documented in Qualtrax. All documentation supporting validation must be readily available to each analyst who uses it.

Training is not necessary when the validation evaluates a minor modification to an existing method[10], or when the new method is substantially similar to an existing method[11].

For validations conducted outside of the laboratory, individuals will be trained appropriately prior to use in casework and this training shall be documented in Qualtrax.

7.2.2.2 CHANGES TO VALIDATED METHODS

Any change[12] to a validated method requires an evaluation of the effect of the change. If the change affects the original validation, a new validation will be performed (to the extent necessary to demonstrate method validity).

7.2.2.3 RELEVANCE TO NEEDS

The performance characteristics of successfully-validated methods will be appropriate and relevant to the customer's needs and consistent with any specified requirements.

7.2.2.4 VALIDATION RECORDS

After the validation has been completed, a validation summary will be prepared by the personnel involved in the validation process. This will include:

- The procedure used for the validation

[10] For example, one that does not meaningfully modify the method procedure or data interpretation
[11] For example, an LC-MS method which has different analytes, but the same methodology
[12] Including changes to data analysis and interpretation

- Specification of the requirements
- Determination of the applicable performance characteristics of the method[13]
- The results obtained
- A statement as to whether the method is fit for the intended use

The validation summary will be reviewed and approved by the Section Chief, Quality Assurance Manager, Technical Leader (when applicable), Assistant Director (or designee), and Director. The Quality Manual shall be updated appropriately.

7.3 SAMPLING

7.3.1 GENERAL

The Forensic Toxicology Section complies with the lab-wide policy regarding sampling.

There is an assumption of homogeneity in toxicology specimens which obviates the requirement for a sampling plan or a sample selection policy. Nonetheless, actions are taken in routine casework to ensure that this assumption is valid. Specimens which may separate (e.g., blood) are inverted to ensure that the specimen is homogeneous.

7.4 HANDLING OF TEST ITEMS

7.4.1 GENERAL

See *ASCL-DOC-01 Quality Manual*.

7.4.1.1 HANDLING PROCEDURES

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.

Evidence is stored in refrigerated storage inside a locked walk-in refrigerator or inside a locked freezer while awaiting analysis at the Little Rock location. 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 if it was received in a sealed state, unless it is in the process of examination, during which time it may remain unsealed.

[13] For example: measurement range, accuracy, measurement uncertainty, limit of detection, limit of quantification, selectivity, linearity, repeatability/reproducibility, robustness against external influences or cross-sensitivity against interference from the matrix of the sample or test object, dilution integrity, bias

A chain of custody transaction is required for all items taken into the custody of an analyst or technician, whether being tested or not, with the exception of items temporarily removed from storage in order to separate test items from them. These items will be placed back into the storage location as soon as practicable.

7.4.1.1.1 STORAGE

See *ASCL-DOC-01 Quality Manual*.

7.4.1.1.2 PACKAGING AND SEALING

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:

- The specimens are brought to the Forensic Toxicology Section
- The specimens are transferred from the submitter to the Forensic Toxicology Section secure storage using the LIMS system
- Each item of evidence is sealed with tape (if initially unsealed)
- The specimens are placed in refrigerated storage

When evidence is transferred form the Evidence Receiving Section the following process occurs:

- An Evidence Receiving Technician retrieves the appropriate specimens from secure storage
- The specimens are transferred from the Evidence Receiving Technician to a section representative using the LIMS
- The specimens are transported to the Forensic Toxicology Section and stored in refrigerated storage

Evidence will be sealed so that the contents cannot readily escape, and 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, including the initials (or other identifier) of the person sealing the evidence across the seal.

Whenever practical, the original seal will be left intact when opening a container. Instead, a new opening will be made to access the evidence. When the analysis (or examination) is complete, this new opening will be properly sealed as outlined in §7.4.2, leaving all original packaging seals intact and clearly marked.

Items with an expectation of frequent analysis may be considered “evidence in the process of examination/analysis” and may be stored unsealed in a limited access area as long as the evidence

is protected from loss, cross-transfer, contamination, and deleterious change. Items should be resealed as soon as practicable. Cases no longer in the process of examination shall be closed and the evidence properly sealed until analysis resumes or a new service request is received.

7.4.1.1.3 CHAIN OF CUSTODY

The chain of custody records the following information for each transfer of evidence, including transfers within and between laboratory premises:

- 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

A chain of custody transaction is required for all transfers of items between persons, locations, or dispositions, for whatever reason, with the exception of grouped items temporarily removed from storage to separate test items from them. In these cases, the remainder of the group must be placed back into storage as soon as is practicable after the intended test items are separated from them.

The LIMS (Laboratory Information Management System) program is normally used to track all transfers of evidence between analysts and other personnel or storage locations including transfers of evidence within and between the Little Rock and Lowell laboratories.

7.4.1.1.4 CUSTOMER NOTIFICATION

Once accepted by the laboratory, the laboratory agrees to test submitted evidence in accordance with laboratory policies and procedures as described in this manual.

By completing and submitting the submission sheet, each customer relinquishes all decisions regarding analytical processing and the choice of methods to the laboratory.

Any testing for which there is not a validated method must be approved in writing by the Chief Forensic Toxicologist, by placing their initials and the date by on the ASCL Evidence Submission Form next to the request.

Before analysis begins, the analyst reviews the request to determine what testing is appropriate. There is no requirement to perform the specific testing requested by the customer on the *ASCL Evidence Submission Form* (ASCL-FORM-12), but the request (and its purpose, if known) guides the decision as to what testing is appropriate.

The Medical Examiner Section is considered an internal customer, and the review of their requests, tenders, and contracts may be performed in a simplified manner. The *Medical Examiner/Forensic Toxicology Section Submission Form* (TOX-FORM-01) contains a detailed list of analysis types, and a

cursory review of the requested testing will be made by the analyst when deciding the course of analysis. No record of this review is necessary.

The actual testing performed for the Medical Examiner Section may differ from the analysis requested on their submission form. If this deviation is routine (e.g., not testing multiple specimen types for volatiles if the blood is negative), then such changes do not require notification of the requesting pathologist. Other, more substantive changes to the requested testing may require notification of the requesting pathologist (or the Chief Medical Examiner if the requesting pathologist is not available).

See §7.8.1.2.2 for additional reporting procedures.

7.4.2 ITEM IDENTIFICATION

Toxicology evidence is routinely subdivided into individual evidence items which are assigned unique identifiers and tracked separately using the chain of custody accessible through the LIMS. Because toxicology specimens are not returned to the submitting agency, or produced in court, the original packaging is routinely destroyed. A description of this packaging must be maintained in the case file, including whether it was found in a sealed state, a description of the marking(s) on the packaging, and any other useful information. Accession (documentation of packaging and sub-itemization) is considered the beginning of analysis for evidence in the Toxicology Section.

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 (if present).

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:

- The specimens are brought to the Forensic Toxicology Section
- The specimens are transferred from the submitter to the Forensic Toxicology Section secure storage using the LIMS
- Each item of evidence is sealed with tape (if initially unsealed)
- The specimens are placed in refrigerated storage

When evidence is transferred from the Evidence Receiving Section the following process occurs:

- An Evidence Receiving Technician retrieves the appropriate specimen from secure storage
- The specimens are transferred from the Evidence Receiving Technician to a section representative using the LIMS
- The specimens are transported to the Forensic Toxicology Section and stored in refrigerated storage

SUB-ITEMIZATION

Toxicology evidence is routinely subdivided into individual evidence items which are assigned unique identifiers and tracked separately using the chain of custody accessible through the LIMS.

Because toxicology specimens are not returned to the submitting agency, or produced in court, the original packaging is routinely destroyed. A description of this packaging must be maintained in the case file, including whether it was found in a sealed state, a description of the marking(s) on the packaging, and any other useful information.

Accession (documentation of packaging and sub-itemization is considered the beginning of analysis for evidence in the Toxicology Section.

7.4.3 DEVIATIONS

During evidence processing, toxicologists and technicians will ensure that any major discrepancies, (i.e., suspect or victim name do not match name listed on sample tubes) are examined and documented. If the toxicologist or technician discovers an inconsistency between the submitted evidence and the submission sheet, or if there is doubt about the suitability of an evidence item for testing, then the analyst shall consult with the customer before proceeding with any testing. Consultation may be by email or phone call (*Agency Contact Form* [ASCL-FORM-06]). Should a response not be received within five business days, the testing may be canceled and specimens placed into long term storage.

For minor inconsistencies, the analyst shall use their judgment on whether to contact the customer, but must make a note of the discrepancy in the case file.

7.4.4 ENVIRONMENTAL CONDITIONS

If environmental conditions are such that the validity or reliability of analytical results could be jeopardized, testing will be stopped until those conditions can be remediated.

7.5 TECHNICAL RECORDS

7.5.1 CASE NOTES

The results worksheet contains the first and last dates of testing. The first date of testing is considered to be the date when the procedure for the first test was started. The last date of testing is considered to be the date when the last test was completed—when the analyst knows that no further analysis is needed to generate the report of laboratory analysis.

The start date for tissue samples where testing begins with homogenization for extraction or volatiles analysis, the start date will be the date of homogenization.

Additionally, much of the data contained in the case record is output from computerized data systems, and contains the date that the data was acquired.

An outline of normal operating parameters (e.g., oven program, gas flow rate) will be kept on the shared Toxicology drive. The date range when each method was in use will be documented in the log book or the appropriate LIMS case file.

When data from multiple cases is recorded on a single printout, kept in a single file, and referenced for all files for which data was generated, the case number for each case for which data was generated will be recorded on the printout. When the printout is placed in each of the appropriate case records, only the individual case number is required.

When more than one analyst assists with an assay, the assisting analyst (or trainee) will initial the data sheets with their initials in parentheses, so that the identity of the analyst responsible for the data is unambiguous.

Data transfers occur when data is collected and transferred to a document (e.g., certified reference material number written onto a batch worksheet). Data transfers, whenever present, will be reviewed as part of batch review and/or case review, as appropriate. The original record of any transferred data will be scanned into the appropriate LIMS folder.

7.5.1.1 TECHNICAL RECORD RETENTION

Case records are stored indefinitely. Quality records (e.g., logbooks) are stored for at least one full accreditation cycle (i.e., four years). The following items are retained for at least eight years:

- Proficiency test records
- Corrective action documentation
- Assessment records
- Training records
- Continuing education documentation
- Court testimony monitoring records

7.5.1.2 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
- AB: antemortem blood
- AF: abdominal fluid
- AM: antemortem
- ATM: acid test mix
- B: base extraction
- BLK: blank
- BK: blank
- BL: bile
- BTM: base test mix
- BR: brain
- CB: cavity blood
- CL: blood clot
- Cont: containing
- Con'td: continued
- CS: cerebrospinal fluid
- CSF: cerebrospinal fluid
- CV: cavity fluid
- DI: deionized
- DNC: did not corroborate
- GS: gastric contents
- HB: heart blood
- HS: heat-sealed
- INSF: insufficient
- ISTD: internal standard
- KD: kidney
- LN: lung
- LV: liver
- ME: manila envelope
- MIP: marked in part
- MS: muscle
- ND: none detected
- NDD: no drugs detected
- Neg: negative
- NL: not labeled
- NR: not reported
- P: page
- PB: peripheral blood
- Pg: page
- PL: pleural fluid
- Pos: positive
- QNS: quantity not sufficient
- RRT: relative retention time
- RT: retention time
- SB: stat blood
- SD: subdural
- SLE: supported liquid extraction
- Soln: solution
- SR: serum
- STC: said to contain
- UB: unknown blood
- UL: unknown liquid
- UR: urine
- VT: vitreous humor
- (curled arrow): containing
- (p with a dot above it): marked in part

7.5.1.3 TECHNICAL RECORD SUFFICIENCY

See *ASCL-DOC-01 Quality Manual*.

7.5.1.4 TECHNICAL RECORD PERMANENCY

An outline of normal operating parameters (e.g., oven program, gas flow rate) will be kept on the shared Toxicology drive. The date range when each method was in use will be documented in the log book or the appropriate LIMS case file.

When data from multiple cases is recorded on a single printout, kept in a single file, and referenced for all files for which data was generated, the case number for each case for which data was generated will be recorded on the printout. When the printout is placed in each of the appropriate case records, only the individual case number is required.

When more than one analyst assists with an assay, the assisting analyst (or trainee) will initial the data sheets with their initials in parentheses, so that the identity of the analyst responsible for the data is unambiguous. Data transfers occur when data is collected and transferred to a document (e.g., certified reference material number written onto a batch worksheet).

Data transfers, whenever present, will be reviewed as part of batch review and/or case review, as appropriate. The original record of any transferred data will be scanned into the appropriate LIMS folder.

7.5.1.5 REJECTION

All cases will be technically and administratively reviewed prior to the release of the report. If the reviewer finds a technical or administrative error, they will then document it on the review form and return the case file to the analyst for correction or in the Reviewer Notes field in the related request in JusticeTrax.

If data, an observation, or a calculation is rejected, the following information will be recorded in the technical record:

- The reason for the rejection
- The identity of the person rejecting
- The date of the rejection

Examples include but are not limited to:

- Multiple injections of positive control for GC-MS screening (BTM)
  - If more than one BTM is shot during a run, it will be indicated on the batch worksheet which positive control is being used for that data set. It should be documented why the other positive controls are not being used (i.e., poor chromatography, relative response failure)
- QA/QC failure
- Contamination of sample blanks

If the reviewer finds a technical or administrative error, they will then document it on the review form and return the case file to the analyst for correction or in the LIMS system under Notes Requester.

If the analyst and the reviewer disagree regarding the error, they should attempt to resolve the issue. If they cannot agree on a solution, then they will meet with the Chief Forensic Toxicologist or Section Quality Manager for resolution.

All manual calculations (e.g., averaging quantitation results, correcting a quantitation value for a non-standard aliquot) in the case record will be checked by the reviewer.

If a correction is required in the imaged case file, the original uncorrected documentation must be maintained in the case file, the correction will be added separately (clearly labeled).

The successful completion of technical and administrative review is recorded by the setting of the appropriate milestone(s) in JusticeTrax.

7.5.2 AMENDMENTS TO TECHNICAL RECORDS

Amendments[14] to technical records must be trackable to previous versions or to original observations. Both the original and amended data/files will be retained, including:

- The date of alteration
- An indication of the altered aspect(s)
- The personnel who made the alteration(s)

Any corrections made to existing hardcopy technical records will be made by an initialed and dated single strikeout (so that what is stricken can still be read) by the person making the change. All additions will be initialed and dated.

The successful completion of technical and administrative review is recorded by the setting of the appropriate milestone(s) in JusticeTrax.

Non-conforming work is subject to the laboratory corrective action policies and procedures.

Stat alcohol or carboximetry results may be released to the Office of the Medical Examiner as preliminary lead information before full technical and administrative review is performed. However, these results must later undergo full technical and administrative review and be reported on the report of laboratory analysis.

Changes made to electronic documents must allow the reviewer to track what changes were made to the document, who made the change, and when. If a correction is made, the original version will be maintained[15].

[14] Including additions, deletions, changes, interlineations, or any other modification to the original information

[15] A second copy of the document is not necessary if it has not yet been placed into JusticeTrax; the correction can be made on the original notes.

Contemporaneous[16] revisions to technical records are not considered to be amendments.

All affected personnel will be notified if the contract needs to be amended after work has begun.

7.6 EVALUATION OF MEASUREMENT UNCERTAINTY

The Forensic Toxicology Section has measurement of uncertainty estimates for the following activities: GC-MS quantitation, LC-MS quantitation, volatiles analysis, and carbon monoxide testing.

Documentation of the calculation of the estimation of the uncertainty of measurement is kept on the shared *Toxicology* drive. Current estimations of the uncertainty of measurement will be documented and maintained in Qualtrax. Uncertainty of measurement for volatiles will be calculated and included on the report while drug quantitation (including carboxyhemoglobin) will not be included on the report. Requests for drug quantitation uncertainty of measurements from external customers will be handled on a case-by-case basis.

Calculations for reporting the estimates of the uncertainty of measurement are handled internally by the reporting software.

7.6.1 UNCERTAINTY COMPONENTS

When constructing the uncertainty budget, all uncertainty components which are of importance in a given situation shall be taken into account. Sources that routinely contribute to the uncertainty in Toxicology include, but are not limited to, the following:

- Reference standards and reference materials (CRMs)
- Methods and equipment (pipettes)
- Measurement process reproducibility
- Glassware including volumetric flask tolerance and serological pipettes
- Control data
- The individuals conducting the measurement (pipette study)

Factors that do not impact the measurement uncertainty to any significant degree (based on previous experience) may be dismissed, but must still be documented.

7.6.1.1 METHODS REQUIREMENTS

Measurements of uncertainty are evaluated for each LC-MS individually, but for GC-MS the budgets will be combined. The measurement uncertainty budget will be re-evaluated at least annually, or as the need arises (e.g., when a significant change occurs in the uncertainty budget). This review/recalculation is required within three months of a change of applicable personnel. Before

[16] Contemporaneous means at the same time. Amendments made after moving on to the next item/task are not considered to be contemporaneous

being used in casework, any new measuring equipment will be evaluated against all affected uncertainty budgets.

The Section Technical Leader and/or Section Chief will be responsible for updating and maintaining MU budgets. Measurement process reproducibility data collected from July 1st through June 30th of the previous year will be used to update appropriate MU budgets. Semi-annual pipette check data collected will also be used to update appropriate MU budgets.

During calculations, the evaluator shall not round any components of the calculation before the final determination of the estimated measurement uncertainty. The estimated measurement uncertainty will be rounded up at the appropriate level of significance, rather than rounded down or truncated.

The coverage probability of the expanded uncertainty will be at least 95.45% (i.e., k=2).

Estimates will be reviewed within three months of personnel changes, and updated as necessary. Significant changes to the method (e.g., instrumentation, procedure) necessitate a review–and possibly a recalculation–of the estimate.

7.6.2 CALIBRATION

VOLATILES TESTING

The measurement of uncertainty for volatiles results have been estimated with a coverage factor of k=2 (95.45% certainty). These estimates will vary for each drug, and can be found on the shared Toxicology drive.

To calculate the reporting range, the mean of the volatiles result is multiplied by the estimate of the uncertainty of measurement and the result rounded up at the third decimal place. This result will be used to express the estimated uncertainty of measurement.

For example, if two ethanol measurements have a mean of 0.107 g%, the estimated uncertainty is 0.047, and then it can be calculated as follows: 0.1075 x 0.047 = 0.0050525, which is expressed as ±0.006 g%. The report would therefore read:

Ethanol	0.107 g% (±0.006 g%)

The coverage factor or the range of certainty is listed on the report so that the customer may properly interpret the significance of the estimation of uncertainty of measurement.

No uncertainty of measurement will be reported for qualitative results, or for results expressed as a "greater than" or "less than" value.

DRUG QUANTITATION

The estimate of the uncertainty of measurement will vary for each drug, and can be found on the shared Toxicology drive and Qualtrax.

To calculate the reporting range, the reported drug quantitation result will be multiplied by the estimate of the uncertainty of measurement and the result rounded up at the second significant figure.

CARBOXYHEMOGLOBIN QUANTITATION

Carboxyhemoglobin saturations will be reported without an estimate of the uncertainty of measurement on the report. This has been determined to be an absolute 4% saturation, not relative to the reported carboxyhemoglobin saturation. This precludes the need for any calculations when reporting a carboxyhemoglobin result.

7.6.3 ESTIMATION PROCEDURE

Reasonable estimation of the performance of the method shall be based on previous experience and validation data. It is important to keep in mind that the nature of certain test methods may preclude a rigorous, metrologically-and statistically-valid calculation of the measurement uncertainty. Only those components under the control of the laboratory need to be considered when estimating the measurement uncertainty. The basic procedure for estimating the measurement uncertainty may include, but is not limited to, the following actions:

- Specify the measurand
- Specify the measurement method, including the equipment or instrument used to take the measurement.
- Construct and document an appropriate uncertainty budget identifying and listing all potential sources of uncertainty, including those not used in the calculation.
- Gather the appropriate measurement data. Sources of measurement data could include method validation, QC data, proficiency tests, replicate testing data, calibration certificates, or scientific literature.
- Estimate the uncertainty of the measurement method in accordance with an appropriate formula.
- Document the estimated uncertainty of the measurement method, and have the results and supporting data readily available in the laboratory.
- Specify calculation and reporting guidelines, including the number of significant figures and/or decimal places in the estimated measurement uncertainty.
- Re-evaluate the estimated measurement uncertainty as scheduled, and as the need arises (e.g., when a significant change occurs in the uncertainty budget).

7.6.3.1 EVALUATION REQUIREMENTS

A measurement uncertainty will be evaluated for all reported quantitative results. This will be available to the customer, either by appearing on the report or by request.

7.6.4 REQUIRED RECORDS

See *ASCL-DOC-01 Quality Manual*.

7.7 ENSURING THE VALIDITY OF RESULTS

7.7.1 GENERAL

The Forensic Toxicology Section uses the quality system outlined in this document to monitor and ensure the quality of its results. Quality control data is used to evaluate the performance of methods and instruments, and to identify trends. Among the policies and procedures which help to ensure high quality test results are:

- The use of certified reference materials and/or internally-generate secondary reference standards
- Use of alternate instrumentation that has been calibrated to provide traceable results
- Functional checks of measuring and testing equipment
- The use of positive and negative controls wherever appropriate, with control charts
- Intermediate checks on measuring equipment
- Technical and administrative review of reported results
- Competency testing of all analysts before they assume casework responsibilities
- Annual proficiency testing in each category of analysis (as possible)
- Use of multiple analytical techniques to confirm positive results
- Use of multiple replicates to confirm quantitative values
- Testimony monitoring (for testifying analysts)

SQCpack software (or equivalent) is utilized to control chart most processes within the Toxicology Section.

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 without the written approval of the Chief Forensic Toxicologist or their designee.

All dilutions of certified reference materials 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. Analysts in the process of training who assist with testing will initial each page of those results, with their initials in parentheses to indicate that they assisted with the testing.

Any urine screens are 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. A log of method/procedure deviations will be kept by the Chief Forensic Toxicologist.

CONTROLS

Positive and negative controls are analyzed as specified in each test method. 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 reference materials are used whenever possible. If no certified reference materials are available, then an uncertified primary reference material (such as a bulk powder or liquid from a chemical supplier) may be used, providing its quality has been verified. If no uncertified primary reference material is available, then tablets or capsules of stated content may be used.

All dilutions of certified reference materials will be made using measured volumetric amounts. The amount of internal standard added to a standard or control should optimally be within an order of magnitude of the amount of analyte in that standard or control.

In a quantitative assay, a control sample will be run if drugs are present in quantities that fall on the appropriate analytical curve.

CALIBRATION CURVES

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/ToxBox used to generate the calibration curve (including the internal standard) will 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 for GC-MS analysis which may be used to record this information.

Calibration curves are generated using a least-square or other well-accepted curve fitting algorithm (e.g., quadratic). The origin will not be used as a data point. The calibration curve will not be forced through the origin. A weighting factor (e.g., $1/x$ or $1/x^2$) may be used if it is demonstrated that this better fits the data. Non-linear or weighted curve fitting may require more calibration points to fully characterize than an unweighted linear calibration. An analysis of residuals may be helpful in this characterization.

The correlation coefficient (r) of the calibration curve must exceed 0.990 and the calculated value of each calibrator must be within 20% of its target value, with the exception of the lowest calibrator, which may vary up to 30% from its target value (as may a control made at this concentration).

If the correlation coefficient is less than 0.990 then the analyst should look for "outliers", where the calibrator 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 calibrators and the outlier is still significantly off of an otherwise linear curve, then this point may be discarded.

If the measured value of a calibrator 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 calibrators, 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(s) on the curve do not have an adequate response to meet quality control criteria, then these point(s) may be discarded. The detection limit of the calibration curve is then raised to the lowest point that meets the quality control criteria.

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:

$$Percent\ difference = \left(\frac{Result - Mean}{Mean}\right) \times 100$$

If a calibration curve does not meet these criteria of acceptability, then this calibration curve is invalid and may not be used to generate quantitative results. The analysis may, however, be used to generate qualitative results—if the appropriate quality control requirements are met.

Ion ratios (Q1/Q) of the calibrators may change within the calibration curve in an ascending or descending manner due to low responses of the ions. Control and casework ion ratios may be set individually to the closest calibrator concentration if needed.

NOTES

Standard abbreviations will be used for common specimen types (see the list of standard abbreviations in § 7.5.1.2 of this manual). 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 is 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 (including the amount of specimen and diluent used) must be included in the case notes.

Batch worksheets are used to document the traceability of certified reference materials and measurement equipment. Micropipettes used for measurements which can have a significant effect on a reported result will be identified on the batch worksheet or the results worksheet.

Certified reference materials used in an assay will be identified on the batch worksheet or the results worksheet. Batch worksheets are also used to record the evaluation of control results, if those controls are not otherwise contained in the case file. This evaluation is performed by a second qualified analyst. Batch worksheets, and the control data associated with them, are stored in the LIMS in case files dedicated to this purpose.

ADDITIONAL ALIQUOTS

If a 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 case where there is no information concerning these levels.

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.

7.7.1.1 VERIFICATION

The Forensic Toxicology Section does not perform verification of independent examinations.

7.7.1.2 CASE REVIEW

All cases will be technically and administratively reviewed prior to the release of the report. No analyst can review their own work product. The review process must confirm that electronic versions of all necessary documentation are in the imaging module of LIMS. Case review documentation may be recorded on the *Forensic Toxicology Case Review Form* (TOX-FORM-09) or in the Reviewer Notes field in the related request in JusticeTrax.

All rejections will be handled according to §7.5.1.5 (Rejections). All non-conforming work identified during review will be handled according to §8.7 (Corrective Action).

The successful completion of technical and administrative review is recorded by the setting of the appropriate milestone(s) in JusticeTrax.

7.7.1.2.1 TECHNICAL REVIEW

See *ASCL-DOC-01 Quality Manual*.

7.7.1.2.2 ADMINISTRATIVE REVIEW

See *ASCL-DOC-01 Quality Manual*.

7.7.1.2.3 TESTIMONY REVIEW

Testimony must be technically reviewed by a competency-tested and authorized reviewer. This can be achieved in multiple ways, including:

- Direct observation of the testimony
- Review of transcripts of testimony given by an examiner

A Testimony Evaluation Form (ASCL-FORM-04 or ASCL-FORM-04_PDF) will be completed by the reviewer, and signed by both the analyst and their supervisor. Feedback shall be given, both positive and in any area needing improvement. If the evaluation is less than satisfactory, the Chief Forensic Toxicologist will determine whether remedial actions are required, which may include the following:

- Re-training, including a mock trial
- Courtroom monitoring by the Chief Forensic Toxicologist for a designated period of time

Testimony review of each testifying analyst/examiner by a competency-tested and authorized reviewer shall occur at least once per accreditation cycle, when practicable. If this review is not practicable, a memorandum will be generated detailing the reason(s). This documentation will be maintained in Qualtrax on the Personnel tab.

Feedback on testimony is also solicited from court (or other) personnel using a Testimony Evaluation Form. This review shall occur at least once annually, when practicable, for each testifying analyst/examiner. If this review is not practicable, a memorandum will be generated detailing the reason(s). This documentation will be maintained in Qualtrax on the Personnel tab.

7.7.2 INTERLABORATORY COMPARISONS

See *ASCL-DOC-01 Quality Manual*.

7.7.2.1 EXTERNAL PROFICIENCY TESTING

For each calendar year, the Forensic Toxicology Section participates in at least one external proficiency test for each discipline in which accredited services are provided.

7.7.3 MONITORING ACTIVITY ANALYSIS

The data from monitoring activities is evaluated as part of the quality control system of the laboratory. When this data is found to be outside acceptable criteria, planned action shall be taken to correct the problem and to prevent incorrect results from being reported. The initiation of the corrective action process may be necessary (see § 8.7).

7.7.4 INDIVIDUAL PROFICIENCY TESTING

Each analyst will be tested at least once in each category of testing in which they perform casework during every five-year period. In the Forensic Toxicology Section these categories are:

- Qualitative
- Quantitative

The Forensic Toxicology Section will complete an external proficiency test from an approved provider in each of these categories of testing annually.

7.7.5 PROFICIENCY TESTING REQUIREMENTS

Analysis, verification, technical review, and administrative review policies are employed during proficiency testing as they are normally applied to casework, except: all parts of a proficiency test provided by an approved test provider shall be examined as completely as the discipline's procedures allow.

Proficiency tests are 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.

Proficiency test records will be maintained for at least fifteen years.

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.

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 (typically ±30% or two standard deviations for drug quantitation and ±10% or two standard deviations for alcohol quantitation, whichever is greater).

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).

If the results are deemed to be unsatisfactory, a Quality Assurance Concern workflow must be initiated.

If the expected result is not attained during any performance monitoring activity, the Quality Assurance Manager will be notified immediately. ANAB requires notification with 30 days when this occurs.

7.7.6 PROFICIENCY TEST SCHEDULE

Each analyst will be proficiency tested at least annually. Each analyst will be tested at least once in each category of testing in which they perform casework during every five-year period.

7.7.7 PROFICIENCY TEST SOURCING

Among approved external proficiency test providers are the College of American Pathologists (FTC proficiency) and Collaborative Testing Services.

Internal proficiency testing is acceptable if approved external proficiency tests have been completed. The Chief Forensic Toxicologist (or 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.

7.7.8 PROFICIENCY TEST RECORDS

See *ASCL-DOC-01 Quality Manual*.

7.8 REPORTING OF RESULTS

7.8.1 GENERAL

The Forensic Toxicology Section complies with the lab-wide policy regarding reporting analytical results.

7.8.1.1 REVIEW AND AUTHORIZATION OF RESULTS

All results will be reviewed and authorized before release.

7.8.1.1.1 DOCUMENTATION

Both the review of results and authorization of results are performed by the author of the report, and are documented by the setting of the draft complete milestone.

Analysts issuing a report based on examination records generated by another Toxicologist shall complete and document a review of all relevant pages of documentation in the case record by initialing the statement on the *Analytical Results Worksheet* (TOX-FORM-03).

7.8.1.2 REPORTS

Toxicology testing is not subject to a sampling plan. Homogeneity is assumed within toxicology specimens.

Each result will be listed on the report related to a source (usually a person), a specimen type (when known), and the type of testing that produced the analytical result.

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 carboxyhemoglobin testing and technical review of that data.

Quantitative results which are above the highest calibrator, but within the acceptable error for this point (i.e., 20% for drug assays, 10% for volatiles) may be reported as that value. Results above that must be reported as “greater than” the highest calibrator (or reanalyzed to bring them into the calibration range).

Quantitative results below the lowest calibrator, but within the acceptable error for this point (i.e., 30% for drug assays [GC-MS quantitation], 10% for volatiles) may be reported as that value. Results below that must be reported as “less than” the lowest calibrator (or reanalyzed to bring them into the calibration range).

For LC-MS quantitation the LOD and LOQ will be administratively set. Values below the LOD and LOQ are typically not reported. Exceptions may be made by the Chief Forensic Toxicologist based on the data and the needs of the case.

When confirmation testing is performed, and a report of initial testing has not been issued, the immunoassay results should be reported unless they are contradicted by the more specific confirmation testing.

If testing for a specific analyte is requested, then that analyte should be addressed on the report. However, this is not necessary if these specific analytes are routinely requested by the submitting agency without regard to the facts of a given case (i.e., a standard list of analytes).

LANGUAGE

Immunoassay results are 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 is disclaimed on the report.

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

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.

If at least one standard-sized aliquot is used in the quantitation of a given drug, then no disclaimer is required for that drug if smaller aliquots are used for corroboration of the quantitative amount.

Examples of common disclaimers are listed below:

Situation	**Disclaimer**
Initial quantitative values are uncorroborated for any reason	The reported drug amount has not been corroborated by replicate analysis.
No certified reference materials are available upon which to base the results of an assay.	No certified reference material was available for this analyte. The reported drug amount is based upon a standard of uncertified purity.
Other incomplete testing due to lack of sufficient sample.	Insufficient sample is available for normal testing.
The sample size used for analysis was less than required but was available for use.	This assay has an increased detection limit.

Reported immunoassays without any further confirmation of any positive immunoassay class 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.

When confirmatory testing has not been completed for all positive immunoassay classes the following disclaimer will be used:

Note: Complete confirmatory testing has not been performed for the positive immunoassay drug class(es): [list]

Specimen type will be specified when needed for clarity.

If THC confirmatory testing has been performed the following disclaimer may be used in lieu of the previous confirmatory testing disclaimer:

Note: Sample was analyzed for Cannabidiol, Delta-9 THC, Delta-9 Carboxy THC, and 11-Hydroxy Delta-9 THC only.

Cannabidiol will be included or excluded depending on the needs of the case (e.g., CBD confirmation may not be applicable to blood at this time).

All reported DUI/DWI urine alcohol results will include the disclaimer:

Note: If a urine sample was taken according to Arkansas Department of Health guidelines, the blood alcohol concentration can be estimated by dividing the urine concentration by 1.3.

7.8.1.2.1 REPORT DISTRIBUTION

Reports are normally made available to the customer electronically through JusticeTrax iResults. Email may be used to transmit results to the customer, but the sender must follow the requirements of A. C. A. § 12-12-312 and the policy on Confidentiality of Records (§ 4.13.1.3).

7.8.1.2.2 REPORTING PROCEDURE

Sample types that are submitted for analysis and are tested will be identified on the report. If multiple samples are submitted on a case but are not routinely tested (e.g., blood, urine, and vitreous submitted on a case), then results for samples where testing was not performed will not be listed on the report.

7.8.1.2.3 CALIBRATION

The ASCL does not perform calibration or issue calibration reports.

7.8.1.3 SIMPLIFIED REPORTING

The ASCL, in agreement with its customers, reports in a simplified way. This agreement is documented on the submission form by the customer's signature.

7.8.1.3.1 REPORTED ELEMENTS

A list of the specific report elements included and excluded on reports is available to the customer on the ASCL website. A link to where this list is located on the website is included on the *Evidence Submission Form* (ASCL-FORM-12_WD or ASCL-FORM-63). All elements are documented (when applicable) and available upon customer request.

7.8.2 COMMON REQUIREMENTS FOR REPORTS

7.8.2.1 REPORT ELEMENTS

The Forensic Toxicology Section complies with the lab-wide policy regarding report elements.

7.8.2.2 RESPONSIBILITIES

The ASCL is responsible for the information contained in each report, except where provided by the customer. Any information provided by the customer and included in the results will be clearly identified on the report. If this information can affect the validity of the results, a disclaimer to that effect will be included.

7.8.3 SPECIFIC REQUIREMENTS FOR TEST REPORTS

7.8.3.1 ADDITIONAL STATEMENTS

If necessary for the interpretation of test results, the following statements will be included:

- Information of specific test conditions, such as environmental conditions
- Where relevant, a statement of conformity[17] with requirements/specifications
- Where applicable, the measurement uncertainty[18], when:
  - It is relevant to the validity/application of the test results, or
  - When instructed by the customer, or
  - When the measurement uncertainty affects conformity to a specification limit

Measurement uncertainties for toxicology volatiles values are included in the report.

7.8.3.1.1 STATUTORY REPORTING REQUIREMENTS

The ASCL is under no regulatory or statutory requirement for how to report measurement uncertainty.

7.8.4 SPECIFIC REQUIREMENTS FOR CALIBRATION CERTIFICATES

See *ASCL-DOC-01 Quality Manual*.

7.8.5 REPORTING SAMPLING–SPECIFIC REQUIREMENTS

Please refer to §7.8.3.2.

7.8.6 REPORTING STATEMENTS OF CONFORMITY

The ASCL does not issue statements of conformity.

7.8.7 REPORTING OPINIONS AND INTERPRETATIONS

7.8.7.1 AUTHORIZATION

See *ASCL-DOC-01 Quality Manual*.

7.8.7.2 SCOPE OF OPINIONS/INTERPRETATIONS

See *ASCL-DOC-01 Quality Manual*.

[17] The ASCL does not routinely issue statements of conformity
[18] Presented in the same units as the measurand, or in a term relative to the measurand (e.g., percent)

7.8.7.3 DIALOGUE

When opinions or interpretations are directly communicated by dialogue to a customer, a record of the communication will be retained[19].

7.8.8 AMENDMENTS TO REPORTS

7.8.8.1 IDENTIFYING THE CHANGE(S)

An amended report is necessary if an error is found on the original report (including reports uploaded to iResults). An "amended request" will be created in the LIMS and all administrative and examination records for the amended analysis will be added to the electronic case record. Administrative and technical reviews are required before an amended report is issued. When an amended report is necessitated by a change in analytical results, then the Section Chief or Section Quality Manager will perform the technical review on the amended request. Documentation of this review will be incorporated into the original case file.

When an amended report is issued, any change of information will be clearly identified. Where appropriate, the reason for the change will be included in the report.

7.8.8.2 STYLE OF AMENDMENT

See *ASCL-DOC-01 Quality Manual*.

7.8.8.3 IDENTIFYING THE AMENDED REPORT

The statement "*AMENDED REPORT: This corrected report replaces the report dated [DATE}*" (or equivalent) will appear below the header information and above the listing of the evidence and the results[20]. The amended report will contain all of the items on the original report and any amendments.

All original records will remain in the case record.

7.8.9 SUPPLEMENTAL REPORTS

A supplemental report is necessary when additional evidence is received after the original report has been issued, additional requests for analysis are made, or other additional testing is required in a case[21]. A "supplemental request" will be created in the LIMS, and all administrative and examination records for the additional evidence will be added to the electronic case record. Administrative and technical reviews are required before a supplemental report is issued. The

[19] For example, using an *Agency Contact Form* (ASCL-FORM-06)
[20] The date of the original report must be entered in the "additional data" tab of the amended request.
[21] When additional evidence is received on a case that has not been completed, the additional evidence may be analyzed an included in the original report.

statement “SUPPLEMENTAL REPORT: This report contains results of additional testing, supplementing the report dated [DATE]” (or equivalent) will appear below the header information and above the listing of the evidence and the results[22]. The supplemental report will contain the updated information from the additional analysis. All original records will remain in the case record.

7.9 COMPLAINTS

The Forensic Toxicology Section complies with the lab-wide policy regarding complaints.

7.10 NONCONFORMING WORK

The Forensic Toxicology Section complies with the lab-wide policy regarding nonconforming work.

7.11 CONTROL OF DATA AND INFORMATION MANAGEMENT

The Forensic Toxicology Section complies with the lab-wide policy regarding control of data and information management.

[22] The date of the original report must be entered in the “additional data” tab of the supplemental request

8 MANAGEMENT SYSTEM REQUIREMENTS

8.1 OPTIONS

8.1.1 GENERAL

The ASCL has a management system capable of supporting and demonstrating the consistent achievement of all accreditation requirements and assuring the quality of laboratory results.

8.1.2 OPTION A

The ASCL opts for Option A, and addresses the following topics:

- Management system documentation
- Control of management system documents
- Control of records
- Actions to address risks and opportunities
- Improvement
- Corrective actions
- Internal audits
- Management reviews

8.1.3 OPTION B

The ASCL is not accredited to ISO 9001, and does not opt for Option B.

8.2 MANAGEMENT SYSTEM DOCUMENTATION (OPTION A)

8.2.1 POLICIES AND OBJECTIVES

The *ASCL Quality Manual* (ASCL-DOC-01) outlines the policies and procedures under which the laboratory operates. This manual acts as a set of supplemental policies and procedures required to competently perform testing in the Forensic Toxicology Discipline at the Arkansas State Crime Laboratory.

When the section policy does not differ from the labwide policy in any significant manner, the reader will be referred to the *ASCL Quality Manual* for the policy. Where there are additional policies and/or procedures, clarifications, or another basis for further information, then that will be included in this document.

8.2.1.1 REQUIREMENT FOR WRITTEN EVIDENCE

Where a form of one of the following words is used in the accreditation requirements, the requirement will be addressed in writing:

- Agree
- Appoint
- Authorize
- Define
- Instruction
- Method
- Plan
- Procedure
- Program/programme
- Record
- Schedule
- Specify

8.2.2 MISSION AND QUALITY POLICY STATEMENTS

See *ASCL-DOC-01 Quality Manual* for the Mission and Quality Policy Statements.

8.2.3 COMMITMENT TO MANAGEMENT SYSTEM

See *ASCL-DOC-01 Quality Manual*.

8.2.4 DOCUMENTATION

See *ASCL-DOC-01 Quality Manual*.

8.2.5 ACCESSIBILITY

See *ASCL-DOC-01 Quality Manual*.

8.3 CONTROL OF MANAGEMENT SYSTEM DOCUMENTS (OPTION A)

8.3.1 CONTROLLED DOCUMENTS

The Forensic Toxicology Section complies with the lab-wide policy regarding document control.

All controlled documents will be available wherever work is performed. Qualtrax is available to any Forensic Toxicology Section user on any computer on the labwide network.

8.3.2 CONTROLLED DOCUMENT POLICIES AND PROCEDURES

8.3.2.1 DOCUMENT APPROVAL

All discipline-specific documents will be prepared by personnel having adequate expertise in the subject. The preparer will be responsible for:

- Preparing the document on the proper format
- Ensuring that document is complete and unambiguous
- Addressing comments from reviewers

The Section Chief will then approve the document. The Section Chief is responsible for:

- Reviewing all discipline-specific controlled documents for:
  - Content
  - Scientific suitability
  - Compliance with labwide policies and procedures
- Approving all discipline-specific controlled documents

After approval, the QA Manager will review the documents for compliance to labwide policies and procedures and approve them.

8.3.2.2 DOCUMENT REVIEW

All controlled documents will be reviewed at least annually. Documents that have been edited within the last year will not require an additional review. This document review will be tracked in Qualtrax.

8.3.2.3 DOCUMENT REVISION

See *ASCL-DOC-01 Quality Manual*.

8.3.2.4 DOCUMENT AVAILABILITY

Qualtrax contains the official version of all controlled documents. Unofficial copies of controlled documents may be made for personal use, but care must be taken to ensure that the most current revision is used. Each copy of these documents will contain the revision date so that the status of the document can be determined.

8.3.2.5 DOCUMENT IDENTIFICATION

See *ASCL-DOC-01 Quality Manual*.

8.3.2.6 DOCUMENT OBSOLESCENCE

Employees will destroy outdated documents when new revisions become available, or clearly mark them as obsolete. It is the employee's responsibility to ensure that they are using the current revision of any controlled document. Any change to a Quality Manual, Health and Safety Manual, or Personnel Handbook requires a revision to the document.

8.4 CONTROL OF RECORDS (OPTION A)

8.4.1 RECORDS

All records shall be legible, readily retrievable, and maintained in a manner that prevents damage, deterioration, or loss of the records. The storage location of physical records must be secure and have limited-access.

Records include both quality and technical records. This policy provides procedures and practices for the identification, collection, organization, accessibility, filing, indexing, access, storage, maintenance, and disposal of records.

TECHNICAL RECORDS

Case files will be retained by the Arkansas State Crime Laboratory in either physical or electronic form. The Arkansas State Crime Laboratory uses the JusticeTrax® LIMS-plus software program. All case documentation will be stored electronically. Once reviewed, this electronic version is considered the official case record.

8.4.2 RECORD POLICIES AND PROCEDURES

8.4.2.1 RECORD RETENTION

Technical records (e.g., case records) are maintained in the LIMS. Once reviewed, this becomes the official case record, and will be maintained indefinitely.

Quality records (e.g., logbooks) are kept near the instrument to which they are associated, in the laboratory area, or in the office area. Quality records are stored for at least one full accreditation cycle (i.e., four years). The following items are retained for at least eight years:

- Proficiency test records
- Corrective action documentation
- Assessment records

- Training records
- Continuing education documentation
- Court testimony monitoring records

The results worksheet contains the first and last dates of testing. The first date of testing is considered to be the date when the procedure for the first test was started. The last date of testing is considered to be the date when the last test was completed-when the analyst knows that no further analysis is needed to generate the report of laboratory analysis.

Additionally, much of the data contained in the case record is output from computerized data systems, and contains the date that the data was acquired.

8.4.2.2 CONFIDENTIALITY

Case records are maintained in the LIMS, which requires a username and password to access. The confidentiality of records is governed by A. C. A. §12-12-312. The scope of covered material includes any records, files, and information kept, obtained, or retained by the laboratory.

Security of case records are maintained by LIMS, which requires a username and password to access. The confidentiality of records is governed by A.C.A. §12-12-312.

Access rights to the LIMS are determined by management, and are limited to those employees who require access to perform their job functions.

8.5 ACTIONS TO ADDRESS RISKS AND OPPORTUNITIES (OPTION A)

8.5.1 RISKS AND OPPORTUNITIES

See *ASCL-DOC-01 Quality Manual*.

8.5.1.1 HEALTH AND SAFETY

See *ASCL-DOC-01 Quality Manual*.

8.5.2 PLANNING

Preventive actions are submitted and implemented using the *Quality Assurance Concern* workflow.

Any preventive actions that are approved will be put into place, and will be placed into the management system[23] when appropriate. The effectiveness of the preventive action will be evaluated as part of the *Quality Assurance Concern* workflow, and reviewed during management review.

[23] For example, into a quality manual, training manual, or test method

8.5.3 PROPORTIONALITY

The actions taken to address risks and opportunities will be proportional to their potential impact on the validity of laboratory results.

8.6 IMPROVEMENT (OPTION A)

8.6.1 IMPROVEMENT

See *ASCL-DOC-01 Quality Manual*. The Forensic Toxicology Section strives to continually improve the effectiveness of its quality management system. To this end, the following activities are planned:

- An annual review of the quality management system
- Annual internal or external assessments
- A consideration of employee suggestions
- Evaluation of our work product through full technical and administrative review of all case files
- Evaluation of any received customer survey comments

8.6.2 EXTERNAL FEEDBACK

See *ASCL-DOC-01 Quality Manual*.

8.7 CORRECTIVE ACTIONS (OPTION A)

8.7.1 NONCONFORMITIES

See *ASCL-DOC-01 Quality Manual*.

8.7.2 PROPORITIONALITY

See *ASCL-DOC-01 Quality Manual*.

8.7.3 RECORDS

See *ASCL-DOC-01 Quality Manual*.

8.8 INTERNAL AUDITS (OPTION A)

The Forensic Toxicology Section complies with the lab-wide policy regarding Internal Audits.

8.9 MANAGEMENT REVIEW

The Forensic Toxicology Section complies with the lab-wide policy regarding Management Review.

9 TEST METHODS

Personnel who authorize results or express opinions/interpretations in the Toxicology will meet the educational requires listed in the *ASCL-DOC-01 Quality Manual*, §9.1. Standard analytical procedures are important to 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.

9.1 METHOD SPECIFIC REQUIREMENTS

GAS CHROMATOGRAPHY (GC)

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 ±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 ±2% around the peak of interest.

The retention time of an analyte may not differ more than ±2% from the retention time of its control. The retention time of a symmetric peak is judged by the retention time at its apex. The retention time of an asymmetric peak may alternately be judged by the retention time of the beginning of the peak. The retention time of the control must be evaluated in the same manner as the analyte.

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 may be changed without regeneration of the method if a known standard of that analyte is run to determine the new retention time. Alternately, the flow and or pressure may be altered to bring the retention time(s) back to their expected value(s).

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.

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 may not differ more than 20%, or the amount specified in the method. At least one SIM qualifier ion must be present.

In a scan mode or selected ion monitoring (SIM) quantitation, the ratio of the qualifier ion(s) to the quantitation ion is measured for each peak. This ratio for an unknown peak is compared to that of one or more positive controls to evaluate whether the analyte in question meets specifications. This ratio can differ by up to 20% relative to the expected ratio (or the amount specified in the method).

The expected ratio can vary with concentration, and can be determined in one of several ways:

- One positive control/calibrator can be selected as representative of the expected value
- If the values in the population of positive controls/calibrators vary too much to pick a single representative value, the ratio from the valid positive control/calibrator closest in concentration to the sample in question may be used.
- The mean of all valid controls/calibrators can be used.

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. 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.

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 ±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. Deuterated compounds may always be used as an internal standard for their un-deuterated version with qualitative testing only.

CARBOXIMETRY

By UV/Visible spectrometry, a matrix blank cannot contain more than 10% 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.

URINE IMMUNOASSAY

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.

RANDOX

There are critical and noncritical error codes generated by the Randox Evidence Investigator. Any critical error codes should be investigated and corrected if possible (e.g., 6037 too many background spots, 6010 chip not processed). Noncritical error codes include warnings of the reading being out of the calibration range (above/below curve).

MEASUREMENTS

Measurements must be performed so as to minimize any errors.

MICROPIPETTING

When micropipetting liquids with a manual micropipette, two methods are acceptable:

Traditional method

1) Set and lock desired volume to be pipetted
2) Firmly attach the pipette tip
3) Press plunger to the first stop only
4) Holding the micropipette vertically (±20°), immerse the pipette tip into the source liquid and slowly return the plunger to its starting position
5) Remove the pipette from the source liquid and place in its target container, placing the pipette tip against the side of the container
6) Slowly press the plunger past the first stop to the bottom of the piston stroke, waiting for the liquid to fully transfer
7) Remove the pipette from the target container and eject the pipette tip into an appropriate waste container

Reverse pipette method

1) Set and lock desired volume to be pipetted
2) Firmly attach the pipette tip
3) Press plunger past the first stop to the bottom of the piston stroke
4) Holding the micropipette vertically (±20°), immerse the pipette tip into the source liquid and slowly return the plunger to its starting position
5) Remove the pipette from the source liquid and place in its target container placing the pipette tip against the side of the container
6) Slowly press the plunger to the first stop only, waiting for the liquid to fully transfer
7) Remove the pipette from the target container and eject the pipette tip into an appropriate waste container

MASSING

All materials to be massed must be placed into a clean receptacle.

DILUTION

Dilution may be performed by using a volumetric flask, filling so as the meniscus coincides with the mark on the neck of the flask. Dilution may be performed by adding measured volumes of liquid by micropipette if (and only if) all measured volumes are of the same solvent. Measured volumes of different solvents may not be additive.

CALCULATIONS

All calculations will be performed and documented in the following manner:

- All decimal places will be carried throughout the calculation
  - Only the result will be adjusted to the proper number of significant figures
  - The reported amount may have fewer significant figures than the calculated result, but not more
- When averaging: if the final digit is 5, then round away from zero (round positive numbers up and negative numbers down)
- When a manually-calculated result is given, the numbers used to generate that result will be explicitly listed if any deviation from normal procedure occurs (e.g., a value is excluded when averaging)

9.2 INDIKO PLUS IMMUNOASSAY SCREEN

Scope

The Indiko Plus is based upon the Enzyme Multiplied Immunoassay Technique (EMIT). This method is applicable to urine. The specimen size is determined by the number of analytes chosen for analysis.

Reagents

- Indiko reagent packs
- Deionized water

Controls

- Indiko controls

Equipment

- Disposable transfer pipettes
- Cuvettes
- Sample cups

Instrumentation

- Thermo Fisher Indiko Plus

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) 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 and specimen ID
4) Place results in appropriate case files

Quality Assurance, Interpretation, Precautions, and Notes

A calibration curve for each assay is generated (at least) weekly before use. Positive controls (above and below the decision point), as well as a negative control (consisting of drug-free urine), are analyzed daily to ensure proper functioning of the instrument.

A control chart is generated (using SQC pack, or an equivalent control charting software) to look for issues or trends in the performance of the controls.

The identity of each specimen must be verified by the analyst with a one-to-one comparison between the specimen labeling and the specimen location entered into the instrument.

Washing solution provided by the manufacturer is consumed at the end of a run to place the instrument in standby mode. This solution is solely used to flush and decontaminate tubing lines and therefore is not subject to expiration.

The results of an immunoassay screen are reported as “positive” or “negative”, dependent upon a comparison of the response of the instrument to an internal calibration curve. Responses more positive than the decision point are considered “positive”. 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.

Preparation of Materials

Materials are purchased fully prepared.

9.3 ETHANOL ANALYSIS

Scope

This method is designed to detect the presence of ethanol and other volatiles in various samples by headspace gas chromatography using dual column analysis. Ethanol is identified by retention time.

This procedure is appropriate for blood, bile, urine, gastric, vitreous, other liquids, and clot samples.

Chemicals and Reagents

- 0.05 % n-propyl alcohol v/v in deionized water
- Methanol
- Isopropanol
- Acetone
- Diflurorethane (if needed)
- Tetrafluoroethane (if needed)
- Toluene (if needed)
- Propane (if needed)

Controls

- Ethanol certified reference materials: 0.05 and 0.20 g%
- Multicomponent volatiles reference material: 0.10 g%
- Calibration curve ethanol, methanol, acetone, and isopropanol standards: 0.010, 0.020, 0.050, 0.100, 0.200, 0.300, 0.400 g%
- Methanol, acetone, and isopropanol control sample
- Difluoroethane, tetrafluoroethane, toluene, and propane control samples (if needed)

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:	HP6890 or equivalent
Headspace unit:	HP G1888 or equivalent
Column type:	Rtx-BAC1: 30 m, 0.32 mm ID, 1.8 µm film thickness
Rtx-BAC2:	30 m, 0.32 mm ID, 1.2 µm film thickness

Instrument Conditions

Gas Chromatograph	
Column	
Carrier:	Constant flow
Flow (mL/min):	~8.0
Inlet	
Inlet temp (°C):	250
Inlet pressure (psi)	27.267
Mode:	Split
Split ratio:	5:1
Detector	
FID temp (°C):	250
Hydrogen flow (mL/min):	40
Air flow (mL/min):	450
Makeup to (mL/min):	45

Headspace Unit	
Temperatures	
Oven temp (°C):	70
Loop temp (°C):	115
Transfer line temp (°C):	120
Times (min)	
Vial equilibration:	3.0
Pressurization:	0.20
Loop fill:	0.05
Loop equilibration:	0.20
Injection:	0.25
GC cycle:	6.5
Pressures (kPa)	
Carrier gas	68
Vial	7

Temperature Ramp

Rate (°C/min)	**Temperature (°C)**	**Hold Time (min)**
	50	1
40	140	0.25

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, Interpretation, Precautions, and Notes

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 in each batch. A control sample must be run for each analyte reported. Positive controls for ethanol will be run at 0.050 g% and 0.200 g%. Positive controls for methanol, acetone, and isopropanol will be run at 0.100 g%. Additional controls may be analyzed as needed. 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) to report quantitative results for an analyte. Qualitative results may be reported based upon retention time alone.

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 blank blood must be analyzed in every batch. Any positive result requires further investigation and appropriate action.

The performance of the controls will be evaluated by a second analyst, and a record of this evaluation will be recorded on a batch worksheet and maintained in the case record.

Alcohols (including Medical Examiner specimens of urine and vitreous, dilutions, and additional specimens) will be run in two batches on two separate instruments whenever practicable. The first batch contains all of the samples to be analyzed. The second batch, which is aliquoted separately, contains those samples which require corroboration. Stat samples from the Medical Examiner's Office and samples requiring retesting due to non-corroboration will be excluded from this requirement and will be run in duplicate. Samples requiring corroboration include:

- Biological samples containing 0.01 g% or more of a volatile component
  - Biological samples containing less than 0.01 g% of ethanol, methanol, isopropanol, or acetone may be reported as "<0.01 g%" without further corroboration
- Non-biological samples containing any amount of ethanol
- Samples which are positive for a qualitative analyte (e.g., toluene, difluoroethane)

Green-stoppered tubes may not be appropriate for volatiles analysis due to potential interferences (e.g., toluene positives) with data interpretation from the preservative (heparin). If toluene is detected in a specimen with a green stoppered tube it will not be reported as present.

Urine that has not been collected according to Arkansas Department of Health guidelines will reported as qualitative only.

Other volatiles (including difluoroethane, tetrafluoroethane, toluene, and propane) can also be qualitatively determined by this procedure using the proper positive and negative controls and run times.

Decomposed specimens may yield greater variation between duplicate runs than is expected from fresh specimens, and should be followed in sequence by a blank to prevent carryover if needed. Tissue specimens are not routinely analyzed by volatiles analysis due to homogenization of the specimen and current Measurement of Uncertainty budgets.

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

$$Percent\ difference = \left(\frac{Result - Mean}{Mean}\right) \times 100$$

If there are values outside the acceptable range, the analysis is repeated in duplicate. If reanalysis is necessary, all original values are discarded and only the newly-acquired results are used in the calculation of reported results.

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 mean as calculated by the formula:

$$Percent\ difference = \left(\frac{Result - Mean}{Mean}\right) \times 100$$

If there are values outside of the acceptable range, the analysis is repeated in duplicate. If reanalysis is necessary, all original values are discarded and only the newly-acquired results are used in the calculation of reported results. If methanol is present and the specimen was taken post-mortem (as may have been from an embalmed source), a disclaimer such as the following must be added:

Note: Methanol is a common component of embalming fluid

A positive result must show a peak in every run on both columns. The retention time for each analyte must be within specifications set by the program to report an analyte as present.

Each quantitative result is reported as the mean of the two experimental results, rounded at the third decimal place. If this mean does not lie in the calibration range, then the result is reported as "greater than" or "less than" the appropriate calibrator.

Each qualitative result is reported as "present" or "not detected".

Please see §6.5 (Metrological Traceability) for guidelines on reporting those values.

Preparation of Materials

Calibration curve reference materials:

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%):
Measure 127 µL of methanol, 128 µL of isopropanol, 127 µL acetone, and 99.618 mL of deionized water using a calibrated volumetric measuring device, whenever practicable.

0.05% n-propyl alcohol v/v in deionized water:
Add 1 mL n-propyl alcohol to 2 L deionized water and mix well.

9.4 RANDOX (EVIDENCE INVESTIGATOR)

Scope

The Randox Biochip Array Technology (BAT) is an immunoassay testing platform allowing for the simultaneous multi-analyte testing in blood and urine samples using Randox Evidence Investigator analyzer. This method is applicable to blood and urine. A 50 µL blood sample (diluted) is typically used for screening while urine requires 10 µL of sample.

Theory

The Biochip is a solid-state device containing an array of discrete test regions which contain immobilized antibodies specific to different drugs of abuse (DOA) compound classes (See tables 1 and 2). These biochips are analyzed using Evidence Investigator, which is a semi-automated benchtop analyzer.

Drugs of Abuse Ultra Whole Blood (DOA ULTRA WB) and Drugs of Abuse Ultra Urine (DOA ULTRA URN) assays are a competitive chemiluminescent immunoassays designed for the semi-quantitative determination of the parent molecule and metabolites of drugs in blood or urine. The drug in the specimen and drug labelled with horseradish peroxidase (HRP) enzyme are in direct competition for the antibody binding sites. Increased levels of drug in a specimen will lead to reduced binding of drug labelled with HRP and thus a reduction in the chemiluminescent signal emitted.

The light signal generated from each of the discrete test regions (DTRs) on the biochip is simultaneously detected and recorded by a cooled charge coupled device (CCD) camera in the Evidence Investigator. The CCD camera has a sensor that converts incident photons produced in the chemiluminescent reaction into electrons; the light output generated is quantified by the CCD camera.

The amount of chemiluminescent signal emitted during the assay is inversely proportional to the concentration of analyte present in the sample. This analyte concentration is calculated from the calibration curve. The calibration curve is evaluated by using two controls provided by the manufacturer and one negative control. The values obtained are used to indicate the presence or absence of a member of a class of drugs targeted by the antibodies.

Chemicals and Reagents

- Water (reverse osmosis or Millipore)
- DOA ultra assay diluent [provided by manufacturer]
- DOA ultra conjugate solution [provided by manufacturer]
- DOA Ultra Whole Blood sample diluent (DOA ULTRA WB DIL SPE) [provided by manufacturer]
- Signal reagent (LUM-EV841/PX) [provided by manufacturer]
  - Two components, Luminol-EV841 (1 x 10 mL) and peroxide (1 x 10 mL), provided by the manufacturer, when mixed in a 1:1 ratio give the working strength signal reagent.
- Wash buffer [provided by manufacturer]

Calibrators and Controls

- DOA Ultra Whole Blood calibrators (DOA ULTRA WB CAL) [provided by manufacturer]
- DOA Ultra Whole Blood controls (DOA ULTRA WB CONTROLS) [provided by manufacturer]
  - Analyte concentrations for each control are outline in the insert (.pdf file on the included compact disc) provided with control kits
- DOA Ultra Urine calibrators (DOA ULTRA URN CAL) [provided by manufacturer]
- DOA Ultra Urine controls (DOA ULTRA URN CONTROLS) [provided by manufacturer]
  - Analyte concentrations for each control are outline in the insert (.pdf file on the included compact disc) provided with control kits
- Sheep's blood (negative control)
- Blank urine (negative control)

Equipment

- DOA Ultra Whole Blood and urine biochips
- 1.5 mL Eppendorf flex tubes
- Pipets and pipettors
- Randox Biochip carrier handling tray
- Thermoshaker
- Tube rotators
- Wash bottle
- Centrifuge

Instrumentation

- Randox Evidence Investigator analyzer

Table 1. DOA Ultra Whole Blood array cutoff concentrations for case work.

Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)
Oxycodone-1	10	Amphetamine	50	Benzoylecgonine (BE)	50
Oxycodone-2	10	Barbiturates	100	Zolpidem	20
Opiates	8	Benzodiazepines-1	20	Tricyclic Antidepressants (TCA)	100
Generic Opioids	10	Benzodiazepines-2	20	Cannabinoids	7
Dextromethorphan	20	Benzodiazepines-3	20	Tramadol	20
Meprobamate	200	Methadone	20	Fentanyl	2

Methamphetamine	40	Phencyclidine (PCP)	20	Buprenorphine	5

Table 2. DOA Ultra Urine Array cutoff concentrations for case work.

Assay	Cut-off (ng/mL)	Assay	Cut-off (ng/mL)	Assay	Cut-off (ng/mL)
Oxycodone-1	100	Benzodiazepines-2	200	Cannabinoids	30
Oxycodone-2	100	Methadone	300	Tramadol	30
Dextromethorphan	30	Opiate	200	Amphetamine	500
Meprobamate	500	Phencyclidine (PCP)	25	Fentanyl	2
Methamphetamine	500	Benzoylecgonine (BE)	150	Norbuprenorphine	5
Barbiturates	200	Zolpidem	30	Generic Opioids	100
Benzodiazepines-1	200	Tricyclic Antidepressants (TCA)	100		

Procedure for DOA Ultra Whole Blood assay and DOA Ultra Urine assay

1) Remove calibrators and biochips, controls kits, blank blood or urine (negative control), and cases from the refrigerator and allow them to reach room temperature for at least 30 minutes.
2) Insert the disc from the calibrators' kit into CD ROM drive and run the "Updater" program followed by the "Update Concentrations". If new controls kit is used, run the updater from the disc in that kit. Ensure investigator software is closed during this installation.
3) Turn on the analyzer followed by the Evidence Investigator software. The camera in the analyzer will initialize (about 5 minutes) after logging into the software.
4) Label each carrier handle for identification purposes and also for pipetting samples.
5) Click the Sample Entry icon to being a new worklist.
   a) Enter relevant details under the "Add" tab.
   b) Choose the desired testing array (ex. DOA Ultra WB array or DOA Ultra URN array).
   c) Scan barcodes to upload calibrators and controls information.
      Right click and assign the corresponding controls.
6) Add samples to the worklist.
7) If the calibrators are not run, follow step 5 but switch to the sample tab in the software. Scan the kit barcode (first barcode on the sheet) to use the associated calibration curve. Scan control barcodes, and assign using right click. Run both positive controls and one negative control with every batch of case samples.

8) Click Accept Carrier after building the worklist.
9) To define the test profile, select all the samples in the worklist and then right click to choose the Select Profile. Another pop-up window will appear with a Select Profile option. Select the appropriate array to display profile details.
10) Make sure all analytes are selected and then click apply. *When a test profile has been applied to a sample a Tick symbol will appear next to the sample position number on the current worklist.*
11) Prepare each calibrator and control by reconstituting using 1 mL of deionized water. *Ensure that the rubber stopper is not removed entirely during reconstitution.*
12) Place reconstituted calibrators and controls on the rotator for 30 minutes.
13) Turn on the Thermoshaker. Set the operating conditions to 37°C and 330rpm.
14) Prepare blood sample dilutions
    a) Transfer 50 µL case sample to Eppendorf flex tubes, and dilute with 150uL of DOA Ultra Whole Blood sample diluent (DOA ULTRA WB DIL SPE). Centrifuge diluted samples at 13000 rpm for 10 minutes.
15) **FOR BLOOD SAMPLES ONLY:**
    a) To each well in a biochip carrier add:
        i) 120 µL assay diluent (DOA ULTRA DIL-ASY)
        ii) 60 µL diluted blood samples (same volume of calibrators and controls shall be added to appropriate wells)
        iii) 120 µL Conjugate (DOA ULTRA WB CONJ). Mix by gently tapping the edge of the handling tray
16) **FOR URINE SAMPLES ONLY:**
    a) To each well in a biochip carrier add:
        i) 220 µL assay diluent (DOA ULTRA DIL-ASY)
        ii) 10 µL urine samples (same volume of calibrators and controls shall be added to appropriate wells)
        iii) 120 µL Conjugate. Mix by gently tapping the edge of the handling tray
17) Place handling tray with biochip carriers on the Thermoshaker and incubate for 30 minutes at 37°C and 330rpm.
18) While the biochip carriers are incubating prepare signal reagent and wash buffer using the following procedures:
    a) Signal reagent
        i) Each carrier needs 3 mL (1.5 mL each of Luminol and peroxide) of signal reagent. Multiply the volume with number of carriers for the final amount of signal reagent necessary for the assay.
        ii) Aliquot required volume of peroxide into an amber colored bottle then add the same amount of Luminol to peroxide, in that order.
        iii) Mix components by rotator for 15 minutes prior to use. *This solution is stable for four (4) hours at room temperature.*
    b) Wash buffer

i) Wash buffer is provided as a concentrate, which requires dilution prior to use. The dilution factor is 31.25 (i.e., 32 mL of concentrate should be added to 968 mL of deionized water and mixed by inversion)

19) Following incubation, remove the handling tray from the thermoshaker. Discard reagents into the sink by using sharp, flicking action of the handling tray.
20) Immediately carry out 6 quick wash cycles.
    a) For each cycle add approx. 350 µL wash buffer to each well, gently tapping the handling tray to release any reagents trapped below the biochip, and flick to waste with a sharp action. *Take care not to overfill wells during washing in order to reduce potential for well-to-well contamination.*
21) Perform 6 slow wash cycles,
    a) For each cycle gently tapping the handling tray for approximately 10 to 15 seconds, and then leave the biochips to soak in wash buffer for 2 minutes.
22) Since only one carrier can be processed at a time, do not discard the final wash until the carrier is ready to process for results. *No carrier should be left to soak for longer than 30 minutes.*
23) Remove the first carrier to be imaged from the handling tray. Remove wash buffer using a sharp, flicking action and tap the carrier onto a wipe to remove any residual wash buffer (tap carrier gently against palm to dry about three-quarters of each well).
24) Add 250 µL of signal reagent from step 18 a. to each biochip well and cover to protect from light.
25) Incubate for 2 minutes (±10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time.
26) Load carrier (with 10 seconds left of 2 minute incubation) into Evidence Investigator analyzer. Proceed to imaging the carrier using the Investigator software.
27) Repeat steps 19-26 for all remaining carriers.
28) Results are processed automatically using software.

Quality Assurance, interpretation, precautions and notes

CALIBRATORS

Up to nine calibrators will be analyzed to set the linear range of the method. The Evidence Investigator uses a non-linear regression; 4-parameter curve fit method for assay calibration. If one calibrator level fails this is automatically removed from the calculation (up to three points can be removed from a curve).

Calibration curves are automatically evaluated by the instrument comparing the correlation coefficient (r-value) for the curve fit calculated during the analysis with the r-value for the target curve fit provided by the manufacturer. The curve fit (r-value) will be ≥ 0.95 for calibration curves to be considered valid. Valid calibration curves generated from one kit can be used to calculate concentrations of controls and case samples from other kits of same lot.

Calibration must be performed at a minimum weekly. Kits from the same lot may use the same valid calibration curve to help optimize the number of biochip carriers used in sample analysis or in the event of a calibration curve error during processing. Reprocessed curves will be from the same analyst within the same month if at all possible. Deviations from this policy require approval from the Section Chief before reprocessing occurs.

QUALITY CONTROLS

Two positive controls at different concentrations and one negative control will be analyzed with every batch of cases to ensure validity of the calibration curve and proper functioning of the instrument.

Controls cannot be used past expiry date. Control settings are lot specific. Control lot reagents cannot be mixed.

Positive controls are considered acceptable when their responses are within ±3 standard deviations from the target concentration. Lot specific target concentrations and standard deviations are provided by the manufacturer. The results are automatically evaluated. A QC report for all controls must be generated to aid in performance evaluation of the calibration curves and controls. Responses in the negative control shall be below the cutoff values.

One positive control must pass the acceptability criteria provided by the manufacturer to consider an assay valid.

A control chart is generated (using SQC pack or an equivalent control charting software) to detect issues or trends in the performance of the controls.

Other notes and requirements

An assay is considered positive when the response is above the cutoff concentration (see above table 1 for blood samples, and table 2 for urine samples), and negative when the values are below the cutoff.

Calibration and control details must be uploaded prior to performing assay using the CD supplied with every Evidence Investigator kit. Failure to upload these details may result in no calibration run.

Analyst must ensure that the Biochip orientation and the sample identity are noted on the base of the well at the beginning of the assay.

Only blood samples require dilution. DOA Ultra Whole Blood sample diluent (DOA ULTRA WB DIL SPE) provided with the DOA Whole Blood Biochips kit shall only be used to dilute samples.

Results from DOA Ultra Whole Blood assays do not have to be recalculated. The 4-fold dilution is automatically compensated for during results processing.

The performance of calibrators and controls is evaluated by a second analyst, and the evaluation is recorded on a batch worksheet which will be maintained in the case record.

The results of an immunoassay screen are reported as “positive” or “negative”, dependent upon a comparison of the response of the instrument to an internal calibration curve. Responses more positive than the decision point are considered “positive”.

Analogous assays (e.g., Benzo 1, Benzo 2, Benzo 3) may be combined on the final report into Benzodiazepines for simplicity of interpretation of analysis to the customer.

In cases where an assay cannot be confirmed with further analyses (e.g., Benzo 3 targeted to clonazepam/7-amioclonazepam, Opiate targeted to morphine) the following disclaimer will be included in the final report.

Note: Complete confirmatory testing has not been performed for the positive immunoassay drug class(es): [list]

Preparation of Materials

Reagent	Preparation	Stability	Storage conditions
Assay diluent solutions	Ready to use.	Manufacturer’s expiration date	+2 ^{0}C to +8 ^{0}C
DOA ultra conjugate solutions	Ready to use. Protect from light.	Manufacturer’s expiration date	+2 ^{0}C to +8 ^{0}C
Unopened Biochips	Ready to use.	Manufacturer’s expiration date	+2 ^{0}C to +8 ^{0}C
Open Biochips	Ready to use. Repackage biochips in ziplock bags and store in desiccated containers (whenever practicable).	14 days	Room temperature
Reconstituted calibrators and controls	Reconstitute with 1 mL of DI water. Avoid foam formation. Store in original vial.	up to 14 days	+2 °C to +8 °C
		up to 28 days	-18 °C to -24 °C
Working strength signal reagent (1:1 Luminol and Peroxide)	Add 1.5 mL Luminol to 1.5mL peroxide. Each carrier requires 3 mL of this mix. Protect from light.	4 hours	Room temperature
Diluted wash buffer	32 mL of concentrated buffer added to 968 mL of deionized water.	30 days	+2 ^{0}C to +8 ^{0}C

9.5 RANDOX (EVIDENCE+)

Scope

The Randox Biochip Array Technology (BAT) is an immunoassay testing platform allowing for the simultaneous multi-analyte testing in blood and urine samples using Randox Evidence+ analyzer. This method is applicable to blood and urine. A 150 µL blood sample (diluted) is typically used for screening while urine requires 600 µL of sample (undiluted).

Theory

The Biochip is a solid-state device containing an array of discrete test regions which contain immobilized antibodies specific to different drugs of abuse (DOA) compound classes (See Tables 1 and 2). These biochips are analyzed using the Evidence+ analyzer.

Drugs of Abuse Ultra Whole Blood (DOA ULTRA WB) and Drugs of Abuse Ultra Urine (DOA ULTRA URN) assays are competitive chemiluminescent immunoassays designed for the semi-quantitative determination of the parent molecule and metabolites of drugs in blood or urine. The drug in the specimen and drug labelled with horseradish peroxidase (HRP) enzyme are in direct competition for the antibody binding sites. Increased levels of drug in a specimen will lead to reduced binding of drug labelled with HRP and thus a reduction in the chemiluminescent signal emitted.

The light signal generated from each of the discrete test regions (DTRs) on the biochip is simultaneously detected and recorded by a cooled charge coupled device (CCD) camera in the Evidence+. The CCD camera has a sensor that converts incident photons produced in the chemiluminescent reaction into electrons; the light output generated is quantified by the CCD camera.

The amount of chemiluminescent signal emitted during the assay is inversely proportional to the concentration of analyte present in the sample. This analyte concentration is calculated from the calibration curve. The calibration curve is evaluated by using two controls provided by the manufacturer and one negative control. The values obtained are used to indicate the presence or absence of a member of a class of drugs targeted by the antibodies.

Chemicals and Reagents

- Water [reverse osmosis or Millipore]
- DOA ultra assay diluent [provided by manufacturer]
- DOA ultra conjugate solution [provided by manufacturer]
- DOA Ultra Whole Blood sample diluent (DOA ULTRA WB DIL SPE) [provided by manufacturer]
- Signal reagent (LUM-EV841/PX) [provided by manufacturer]
    - Two solutions [Luminol and Peroxide] are combined in a 1:1 ratio by the Evidence+ analyzer
- Wash buffer [provided by the manufacturer]

Calibrators and Controls

- DOA Ultra Whole Blood calibrators (DOA ULTRA WB CAL) [provided by the manufacturer]
- DOA Ultra Whole Blood controls (DOA ULTRA WB CONTROLS) [provided by the manufacturer]
    - Analyte concentrations for each control are outlined in the insert (.pdf file on the included compact disc) provided with the control kits
- DOA Ultra Urine calibrators (DOA ULTRA URN CAL) [provided by the manufacturer]
- DOA Ultra Urine controls (DOA ULTRA URN CONTROLS) [provided by the manufacturer]
    - Analyte concentrations for each control are outlined in the insert (.pdf file on the included compact disc) provided with the control kits
- Sheep's blood (negative control)
- Blank urine (negative control)

Equipment

- DOA Ultra Whole Blood and urine biochips
- 10 mL culture tubes
- Pipettes and tips
- Vortex
- Centrifuge
- Tube rotators

Instrumentation

- Randox Evidence+ Analyzer

Table 1. DOA Ultra Whole Blood array cutoff concentrations for casework.

Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)
Oxycodone 1	10	Amphetamine	50	Benzoylecgonine (BE)	50
Oxycodone 2	10	Barbiturates	100	Zolpidem	20
Opiates	10	Benzodiazepines 1	20	Tricyclic Antidepressants (TCA)	100
Generic Opioids	10	Benzodiazepines 2	20	Cannabinoids	10
Dextromethorphan	20	Benzodiazepines 3	20	Tramadol	20
Meprobamate	200	Methadone	20	Fentanyl	2
Methamphetamine	50	Phencyclidine (PCP)	20	Buprenorphine	5

Table 2. DOA Ultra Urine array cutoff concentrations for casework.

Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)
Oxycodone 1	100	Barbiturates	300	Zolpidem	50
Oxycodone 2	100	Benzodiazepines 1	200	Tricyclic Antidepressants (TCA)	300
Opiates	100	Benzodiazepines 2	200	Cannabinoids	50
Generic Opioids	100	Benzodiazepines 3	200	Tramadol	30
Meprobamate	1000	Methadone	300	Fentanyl	2
Methamphetamine	500	Phencyclidine (PCP)	50	Buprenorphine Metabolite	10
Amphetamine	500	Benzoylecgonine (BE)	150		

Procedure for DOA Ultra Whole Blood and DOA Ultra Urine assays:

1. Remove calibrators, controls, biochips, blank blood or urine, and cases from refrigeration for approximately 30 minutes to allow samples to reach room temperature.
2. Pre-run maintenance
    a. Empty the 2 L and 10 L reservoirs for displacement fluid and wash buffer on the instrument
    b. Replace the 2 L bottles on the instrument. Ensure all tubing is connected securely.
    c. Fill the 10 L reservoirs with DI water. Once full, add an entire bottle of displacement fluid into the reservoir labeled “DISP” and an entire bottle of wash buffer into the reservoir labeled “TBST” on the instrument.
    d. After the 2 L reservoirs have been refilled and primed, turn on the side power switch on the analyzer.
3. Reconstitute calibrators and controls with 1 mL of DI water. Rotate for 30 minutes.
4. Load the calibrator and control CDs to update concentrations.
    a. Deselect “Investigator” controls before applying update.
5. Open “Evidence V4” software and begin initializing the analyzer.
6. Once initialization is complete:
    a. Check luminol and peroxide percentages. Replace bottles if less than 25% volume is remaining.
    b. Ensure carrier chute is empty and the biohazard waste is empty.
    c. Load diluent and conjugate container wedges.
        i. Before loading into the analyzer, ensure no foam exists in container wedges.
        ii. When the light on the instrument is green, open the door and insert wedges.
7. Load cassette sleeve with barcode facing inward. The biochips will be facing up.
8. Remove calibrators and controls from the rotator and transfer into culture tubes. Label with appropriate barcodes provided by the manufacturer.
9. Run calibration curve. (This must be completed before any worklist containing controls or case are submitted.)

a. Select calibration, select calibration entry.
b. Select "DOA Ultra WB Array" for blood. Select "DOA Ultra Urine" for urine.
    i. Do NOT press "Accept".
c. Load left hand ring with calibrators in positions 1-9.
d. Select "Accept"

10. Prepare samples
    a. BLOOD SAMPLES
        i. Pipette 150 µl sample and 450 µL DIL-SPE into a culture tube
        ii. Cap tubes and centrifuge at 3000 rpm for 10 minutes
        iii. Remove caps and place culture tubes in right hand sample ring
    b. URINE SAMPLES
        i. Pipette 600 µL of sample in a culture tube
        ii. Cap tubes and centrifuge at 3000 rpm for 10 minutes
        iii. Remove caps and place in right hand sample ring
11. Analyze controls and samples
    a. Sample entry
        i. Type "F3" to load a pre-built worklist or start a new worklist
        ii. To start a new worklist:
            1. Select Entry type, select list entry
            2. Select Array type, select DOA Ultra WB (UR) Array
            3. Assign sample position (position on rack)
            4. Select Sample Code, assign case number and sample ID
            5. Select and highlight all controls/samples, right click to select "tubes"
            6. Select and highlight all controls/samples, right click to assign profile
                a. DOA Ultra WB ASCL for blood
                b. DOA Ultra Urine ASCL for urine
12. To eject cassettes (Select Maintenance > Service > Eject cassettes)
13. Remove reagents when applicable
    a. Reagents are good for the duration of one kit (approximately one month)
    b. Reagents differ between blood and urine kits
14. Close software and turn off analyzer using the side power switch – Select "no" to automatically turning off PC and analyzer.
15. Empty liquid waste container.

Quality Assurance, interpretation, precautions, and notes

CALIBRATORS

Up to nine calibrators will be analyzed to set the linear range of the method. The Evidence+ uses a non-linear regression; 4-parameter curve fit method for assay calibration. If one calibrator level fails this is automatically removed from the calculation (up to three points can be removed from a curve).

Calibration curves are automatically evaluated by the instrument comparing the correlation coefficient (r-value) for the curve fit calculated during the analysis with the r-value for the target curve fit provided by the manufacturer. The curve fit (r-value) will be ≥ 0.95 for calibration curves to be considered valid. Valid calibration curves generated from one kit can be used to calculate concentrations of controls and case samples from other kits of same lot.

Calibration must be performed at a minimum every 21 days for blood, and with the opening of a new kit. Calibration must be performed at a minimum of every 14 days for urine, and with the opening of a new kit. Kits from the same lot may use the same valid calibration curve[24]. Reprocessed curves generated by the same analyst will be used whenever possible, preferring the most recent curve. Deviations from this policy require approval from the Section Chief before reprocessing occurs.

If calibration on a batch is required, the calibration curve must be analyzed and accepted by the software prior to analyzing positive or negative control and sample submission.

QUALITY CONTROLS

Two positive controls at different concentrations and one negative control will be analyzed with every batch of cases to ensure the validity of the calibration curve and the proper functioning of the instrument.

Controls cannot be used past expiry date. Control settings are lot specific. Control lot reagents cannot be mixed.

Positive controls are considered acceptable when their responses are within ±3 standard deviations[25] from the target concentration. Lot specific target concentrations and standard deviations are provided by the manufacturer. The results are automatically evaluated. A QC report for all controls must be generated to aid in performance evaluation of the calibration curves and controls. Responses in the negative control shall be below the cutoff values.

One positive control must pass the acceptability criteria provided by the manufacturer to consider an assay valid.

A control chart is generated (using SQC pack, or an equivalent control charting software) to look for issues or trends in the performance of the controls.

Other notes and requirements

An assay is considered positive when the response is at or above the cutoff concentration (see Table 1 for blood samples and Table 2 for urine samples), and negative when the values are below the cutoff.

[24] For example, to optimize the number of biochip carriers used, or in the event of a calibration curve error when processing

[25] The standard deviation value is provided by the vendor

Calibration and control details must be uploaded prior to performing the assay, using the CD supplied with every Evidence+ kit. Failure to upload these details may result in no calibration run.

Only blood samples require dilution. Only the DOA Ultra Whole Blood Sample Diluent (DOA ULTRA WB DIL SPE) provided with the DOA Whole Blood Biochips kit shall be used to dilute samples. Results from DOA Ultra Whole Blood assays do not have to be recalculated. The 4-fold dilution is automatically compensated for during results processing.

The performance of calibrators and controls is evaluated by a second analyst, and the evaluation is recorded on a batch worksheet which will be maintained in the case record.

The results of an immunoassay screen are reported as "positive" or "negative", dependent upon a comparison of the response of the instrument to an internal calibration curve. Responses greater than the decision point are considered "positive".

Analogous assays (e.g., Benzo 1, Benzo 2, Benzo 3) may be combined on the final report into Benzodiazepines for simplicity of interpretation of analysis to the customer.

In cases where an assay cannot be confirmed with further analyses (i.e., Benzo 3 targeted to clonazepam/7-amioclonazepam, Opiate targeted to morphine) the following disclaimer, or equivalent, will be included in the final report.

Note: Complete confirmatory testing has not been performed for the positive immunoassay drug class(es): [list]

Preparation of Materials

Reagent	Preparation	Stability	Storage Conditions
Assay diluent solutions	Ready to use	Manufacturer's expiration date	+2°C to +8°C
DOA ultra conjugate solutions	Ready to use. Protect from light.	Manufacturer's expiration date	+2°C to +8°C
Unopened biochips	Ready to use	Manufacturer's expiration date	+2°C to +8°C
Opened biochips	Ready to use. Seal excess biochips and store in dessicator.	14 days	Room temperature
Reconstituted calibrators and controls	Reconstitute with 1 mL of DI water. Store in original vial.	Up to 14 days	+2°C to +8°C
Signal Reagent	Ready to use	Up to 30 days	+2°C to +8°C
Displacement fluid	320mL to 10L of DI water	48 hours	Room temperature
Diluted wash buffer	320mL to 10L of DI water	24 hours	Room temperature

9.6 BASE SCREEN

Scope

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 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.

This method is applicable to urine, blood, bile, tissue homogenates, vitreous humor, and gastric contents. A 5 mL or 5 g sample is generally used for screening, and other sample amounts may be used for quantitation.

Chemicals and Reagents

- Concentrated ammonium hydroxide
- Concentrated hydrochloric acid
- 1N Hydrochloric acid
- N-Butyl chloride (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 (1.0 µg/mL each of amphetamine, phentermine, 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 caps with rubber septa
- Crimper

Instrumentation

Gas chromatograph:	Agilent 6890 or equiv
Mass spectrometer:	Agilent 5973 or equiv
Autosamplier:	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):	4.81 (variable)
Pulse pressure (psi):	20.0
Pulse time (min):	0.50
Purge flow (mL/min):	20.7
Purge time (min):	2.00
Total flow (mL/min):	24.9 (variable)
Gas saver:	On
Saver flow (mL/min):	20.0
Saver time (min):	2.00
Gas type:	Helium

Column	
Mode:	Constant pressure
Pressure (psi):	4.81
Initial flow (mL/min):	1.5
Avg. velocity (cm/sec):	64
Detector	
Detector:	MSD
Transfer line temp (°C):	280
Quad temp (°C):	150
Source temp (°C):	250
Mass range (amu):	35-550, scan mode
Threshold:	150
Number of samples:	2
Solvent delay (min):	2.45 (variable)

Temperature Ramp

Rate (°C/min)	Temperature (°C)	Time (min)
	50	0.5
30	350	2.0

Procedure

1) Label the proper number of 15 mL extraction tubes for the samples and controls to be extracted.
2) Add 50 µL of 0.1 mg/mL methaqualone to each tube.
3) Add 50 µL of base test mix to the positive control.
4) Pipette 5 mL of sample, control, or 5 g 1:1 tissue homogenate (w/w in normal saline) into the labeled tubes.
5) Adjust the pH of each specimen to approximately 9 by adding approximately 200 µL of concentrated ammonium hydroxide.
6) Add approximately 10 mL of n-butyl chloride to each tube, cap tightly and place tubes on rotator for approximately 10-15 minutes, or until extracted.
7) Remove tubes from rotator, place in centrifuge for approximately 5 minutes or until separated.

8) Add approximately 5 mL of 1N hydrochloric acid to each clean labeled tube.
9) Pipette the top layer (n-butyl chloride) from each sample tube into labeled 15 mL 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.
12) Add approximately 100 µL of chloroform to each vial.
13) Cap tightly and repeat the rotation and centrifugation steps.
14) Carefully transfer the bottom (chloroform) layer from each extract to a properly labeled autosampler vial and crimp on the septum cap.
15) 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.
16) Run the sequence, then compare retention times and mass spectra of peaks within the chromatograms to known retention times and mass spectra, if known.

Quality Assurance, Interpretation, Precautions, and Notes

A positive control (base test mix) and a negative control (a matrix blank) are extracted and analyzed with each batch of samples.

Any significant chromatographic problems will 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.

Morphine, cannabinoids, clonazepam, lorazepam, and benzoylecgonine will not generally be detected with this screening procedure. Screening for these compounds can be accomplished by additional methods.

When the presence of imipramine is detected with the metabolite desipramine, neither drug will be reported as present in a given case due to potential analyte degradation on the inlet.

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.

Pressures and flows may be changed as needed to ensure the proper functioning of the method.

The performance of the controls will be evaluated by a second analyst, and a record of this evaluation will be recorded on a batch worksheet and maintained in the case record.

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, phentermine, methamphetamine, diphenhydramine, amitriptyline, nortriptyline, oxycodone, and alprazolam via a calibrated volumetric measuring device, whenever practicable. Make up to 10 mL with methanol.

9.7 ACID SCREEN

Scope

This method is designed to detect acidic/neutral 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 retention time (if known).

This method is applicable to urine, blood, bile, tissue homogenates, vitreous humor, 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 (ACS 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/evaporation apparatus
- Centrifuge
- 13x100 mm culture tubes
- Autosampler vials with inserts and 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
Inlet temp (°C):	250
Pressure (psi):	4.81 (variable)
Pulse pressure (psi):	20.0
Pulse time (min):	0.50
Purge flow (mL/min):	20.7
Purge time (min):	2.00
Total flow (mL/min):	24.9 (variable)
Gas saver:	On
Saver flow (mL/min):	20.0
Saver time (min):	2.00
Gas type:	Helium

Column	
Mode:	Constant pressure
Pressure (psi):	4.81
Initial flow (mL/min):	1.5
Avg. velocity (cm/sec):	64
Detector	
Detector:	MSD
Transfer line temp (°C):	280
Quad temp (°C):	150
Source temp (°C):	250
Mass range (amu):	35-550, scan mode
Threshold:	150
Number of samples:	2
Solvent delay (min):	2.45 (variable)

Temperature Ramp

Rate (°C/min)	Temperature (°C)	Time (min)
	50	0.5
30	200	0
20	330	1.5

Procedure

1) Label the proper number of 15 mL extraction tubes for the samples and controls to be extracted.
2) Add 100 µL pf the barbital internal standard solution to each tube.
3) Add 100 µL of the acid test mix to the positive control.
4) Pipette 5 mL of sample, control, blank, or 5 g 1:1 tissue homogenate (w/w in normal saline) into the labeled tubes.
5) Add approximately 0.2 g potassium phosphate monobasic to each tube.
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 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 approximately 100 µL of 80% ethanol to each tube and vortex or stopper the tube 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 retention time and mass spectra of peaks within the chromatograms to known retention times and mass spectra, if known.

Quality Assurance, Interpretation, Precautions, and Notes

A positive control (acid test mix) and a negative control (a matrix blank) are extracted and analyzed with each batch of samples.

Any significant chromatographic problems will be investigated and appropriate action taken.

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

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.

Instrument pressures and flows may be changed as needed to ensure the proper functioning of the method.

The performance of the controls will be evaluated by a second analyst, and a record of this evaluation will be recorded on a batch worksheet and maintained in the case record.

Preparation of Materials
Barbital stock solution (20 mg/mL):
Add 200 mg barbital with a calibrated volumetric measuring device, whenever practicable, 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 with a calibrated volumetric measuring device, whenever practicable. Make up to 10 mL with methanol.

9.8 GC-MS QUANTITATION

Scope

This method is an adjunct to the qualitative base extraction listed above, designed to add quantitation to the qualitative identification. The analytes are extracted by the appropriate liquid-liquid extraction with concomitantly-extracted calibrators and controls. Urine specimens will not be quantitated, due to the difficulty in determining the relevance of those quantitative results. The needs of the case determine which specimens are quantitated.

The general requirements for quantitation by this method are set out below. There is some necessary variation in the assay specifications due to the differing behaviors and requirements of each targeted analyte. The expected behavior for each analyte can be found in the appropriate validation document.

Instrumentation

Gas chromatograph:	Agilent 7890 or equiv
Mass spectrometer:	Agilent 5975 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):	7.65 (variable)
Pulse pressure (psi):	20.0
Pulse time (min):	0.50
Purge flow (mL/min):	20.7
Purge time (min):	2.00
Total flow (mL/min):	25.4 (variable)
Gas saver:	On
Saver flow (mL/min):	20.0
Saver time (min):	2.00
Gas type:	Helium

Column	
Pressure (psi):	7.65
Initial flow (mL/min):	2.0
Avg. velocity (cm/sec):	73
Detector	
Detector:	MSD
Transfer line temp (°C):	280
Quad temp (°C):	150
Source temp (°C):	250
Mass range (amu):	35-550, scan mode
Threshold:	150
Number of samples:	2
Solvent delay (min):	2.45 (variable)

Temperature Ramp

Rate (°C/min)	Temperature (°C)	Time (min)
	50	0.5
30	200	0
20	330	1.5

Procedure

1) Prepare, for each targeted analyte:
   a) Up to six calibrators, prepared from a certified reference material (CRM)
   b) Two positive controls, from a separate CRM than the calibrators
   c) One negative matrix control
2) Prepare all case specimens.
3) Extract each item using the base extraction procedure.
4) Place vials in autosampler tray and set up a sequence ensuring that a blank is injected before each sample or control, to detect possible carryover from one specimen to the next.
5) Run the sequence using the instrumental parameters listed above.
6) Perform data analysis.

Quality Assurance, Interpretation, Precautions, and Notes

Calibrators:
Up to six calibrators will be analyzed to set the linear range of the method. These calibrators will be prepared in the appropriate matrix using CRMs, whenever available. The concentrations of these calibrators will vary by analyte, with the expected working range listed in the validation document for each analyte. Calibrators outside of the validated working range may not be included in the assay. The use of calibrated analytical balances, pipettes, and volumetric measuring devices, as appropriate, are required for calibrator preparation.

The calibration curve must meet the requirements outlined in §7.7.1, *Calibration Curves*.

Controls:
The test mixes listed in the qualitative methods are replaced in this method by positive controls of the targeted analyte(s). The concentration of the positive controls will vary by analyte, depending upon the expected working range of the assay for that analyte. At least two positive control concentrations will be analyzed, designed to evaluate the lower half and the upper half of the working range. The CRM used to prepare the positive control must be from a different source than the CRM used to prepare the calibrators. Each positive control must fall within 20% of its target value (30% for the concentration of the lowest calibrator) and be within the working range of the calibration curve.

A negative control will be extracted—containing only internal standard(s)—to demonstrate the absence of any contaminant which would result in a false positive response. The targeted analyte must not be detected (reportable) in the negative control.

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

Other notes and requirements:
The qualitative presence of each analyte is determined by an evaluation of the full-scan EI mass spectrum—rather than using ion ratios, as is the case with SIM analysis. Nonetheless, ion ratios are a useful tool in detecting coelution by interfering compounds. The response of each analyte is measured using a quantitation ion.

The response of a second qualifier ion is also measured to determine an ion ratio (the qualifier ion response divided by the quantitation ion response). The qualifier ion ratio is set for the method in the same way as in a SIM analysis. To report a quantitative value, the ion ratio for a targeted analyte must be within 20% of the ion ratio set for the method. If the ion ratio is more than 20% from the ratio set for the method, then the analyte may only be reported qualitatively.

If there is a contribution to the quantitation or qualifier ions from a compound which coelutes with either the internal standard or the targeted analyte, alternate ions may be selected to avoid the contribution from this coeluting compound. These ions must be used throughout the affected quantitation batch.

If the amount of analyte present in an extract saturates (or is expected to saturate) the mass spectrometer detector, the final extract may be diluted in a larger-than-normal amount of solvent to prevent this effect—typically double the normal amount is used (i.e., 200 µL of either chloroform or 80% ethanol, rather than 100 µL).

Multiple analytes may be added to the same calibrators and controls. More than five analytes per vial may lead to solvent saturation issues, and is discouraged.

A 5 mL case aliquot is typically used, but this aliquot size may be altered as necessary to ensure that the obtained result is within the working range of the calibration curve.

Instrument pressures and flows may be changed as needed to ensure the proper functioning of the method.

The performance of the calibrators and controls will be evaluated by a second analyst, and a record of this evaluation will be recorded on a batch worksheet and maintained in the case record.

9.9 MORPHINE AND 6-MONOACETYLMORPHINE WITH MBTFA (LIQUID EXTRACTION)

Scope

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

Chemicals and Reagents

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

Controls

- Certified reference material 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:	ZB-5 or equiv
Length (m):	15
ID (mm):	0.25
Film thickness (µm):	0.25

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):	6.5 (variable)

Temperature Ramp

Rate (°C/min)	Temperature (°C)	Time (min)
	50	0.5
30	200	0
20	330	1.5

Ions Monitored

Morphine:	364, 477, 311
6-Monoacetylmorphine:	364, 423, 311
Nalorphine:	390, 590

Procedure

1) Label the proper number of 15 mL 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 0.01 mg/mL (10 µg/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°
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 311, 364, 390, 423, 477, and 503 throughout the run.

Quality Assurance, Interpretation, Precautions, and Notes

The calibration curve should extend from 0.025 µg/mL to 2 µg/mL for morphine and 0.025 µg/mL to 1.5 µg/mL for 6-monoacetylmorphine. 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 positive control must 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. A negative control (matrix blank) must also be extracted and analyzed with each batch of samples.

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.

Methanol must not be used to rinse the autoinjector syringe because it hydrolyzes the derivatives formed in this procedure. Anhydrous ethyl acetate is 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	Quant Ion	Qualifier Ion 1	Qualifier Ion 2
Morphine	364	477	311
6-Monoacetylmorphine	364	423	311
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. The performance of the calibrators and controls will be evaluated by a second analyst, and a record of this evaluation will be recorded on a batch worksheet and maintained in the case record.

Preparation of Materials

pH 9.1 carbonate buffer:
Add 10.6 g Na2CO3 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.

9.10 CARBON MONOXIDE BY UV-VIS SPECTROMETER

Scope

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.

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, or equivalent.
- 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 (if necessary).
2) Add approximately 2 milligrams of solid sodium hydrosulfite to a cuvette containing approximately 2.5 mL of 0.1% sodium carbonate.
3) Add 10 µL of blood and mix.
4) Add 200 µL 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-A558/A532).
7) Turn off the UV-Visable spectrometer lamps (if necessary).

Quality Assurance, Interpretation, Precautions, and Notes

A positive control and a negative control of blank 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 are run and the results must agree to within ±3% from the mean. The mean of the two results is reported.

The matrix blank cannot contain more than 10% 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 below 10% carboxyhemoglobin saturation are reported as "<10 % saturation". Results above the range of controls must be reported "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.

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. This solution expires after 1 year.

0.1% sodium carbonate:
Add 0.25 g of sodium carbonate to 250 mL of deionized water, mixing well. This solution expires after 1 year.

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

9.11 GAMMA-HYDROXYBUTYRATE (GHB) SCREEN AND QUANTITATION

Scope

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.

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-d6

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

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):	2.45 (variable)

Temperature Ramp

Rate (°C/min)	**Temperature (°C)**	**Time (min)**
	50	0.5
30	200	0
20	330	1.5

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 0.05 mg/mL (50 µg/mL) GHB-d6 internal standard to each tube.
3) Add 500 µL of 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, Interpretation, Precautions, and Notes

The ratio of the qualifier ion(s) relative to the quantitation ion may 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 may not differ more than 2% from the retention time of its control.

Curve points must be run bracketing the concentration of 0.01 mg/mL (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

Positive 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

Suggested calibrator and control preparation:
CALCULATION FROM GHB SODIUM SALT TO FREE BASE

GHBNa-D6: MW/FW = 132.12/109.12 = 1.21 mg/mL (Use this, based on what concentration you want, to figure out how many µL needed)

GHBNa: MW/FW = 126.09/103.09 = 1.22 mg/mL

(Use the calculated concentrations above, and what concentration you want to make, to calculate what volume will be needed)

GHB CALS AND CTRLS PREP

GHB-D6 (50 µg/mL) I.S.

- 410 µL GHBNa-D6 + 9590 µL MeOH

GHB Ctrl (50 µg/mL)

- 410 µL GHBNa + 9590 µL MeOH

GHB Calibrators (10 µg/mL and 100 µg/mL)

- For 100 µg/mL: 820 µL GHBNa + 9180 µL MeOH
- For 10 µg/mL: 1:10 dilution of 100 µL/mL calibrator

A negative control (a urine matrix blank) must also be analyzed with each batch of specimens.

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.

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

The performance of the controls will be evaluated by a second analyst, and a record of this evaluation will be recorded on a batch worksheet and maintained in the case record.

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-d6:
Add 500 µL of a 1 mg/mL certified reference material with a calibrated volumetric measuring device, whenever practicable and make up to 10 mL with methanol.

50 µg/mL GHB sodium salt (81.77 µg/mL GHB) from certified reference material*:*
Add 500 µL of a 1 mg/mL certified reference material with a calibrated volumetric measuring device, whenever practicable and make up to 10 mL with methanol.

100 µg/mL GHB sodium salt (81.77 µg/mL GHB) from powder:
Add 0.0250 g GHB sodium salt with a calibrated volumetric measuring device, whenever practicable and make up to 25 mL with methanol.

9.12 LC-MS SMRM DRUG SCREEN IN BLOOD

Scope

This method is designed to screen and semi-quant members of a targeted list of analytes by tandem liquid chromatography-mass spectrometry scheduled multiple reaction monitoring (LC-MS dMRM) analysis.

This method is validated for use with blood specimens only. Calibrators, controls, and extracted blanks should be made in the appropriate matrix. A 250 µL aliquot is used for analysis.

This method is designed to detect the presence and semi-quantitation of targeted analytes. The analytes are separated from their matrix by supported liquid extraction (SLE), separated from one another by HPLC, and detected by tandem mass spectrometry, monitoring one to two transitions for the analytes and one transition for isotopically-labeled internal standards.

Chemicals and Reagents

- LC-MS grade formic acid (Fisher part A117-50, or equivalent)
- Ultrapure water (17 MΩ-cm or greater)
- Ammonium formate (Fisher part A115-50, or equivalent)
- LC-MS grade methanol (Fisher part A456-4, or equivalent)
- Ammonium hydroxide (Fisher part A470-500, or equivalent)
- HPLC grade ethyl acetate (Fisher part E196-4, or equivalent)
- Sheep blood, horse blood, or human blank blood
- 96-well plate Nunc (Fisher part 12-565-606, or equivalent)
- Isolute SLE+ 400 mg Supported Liquid Extraction Plate (Biotage, or equivalent)

Controls

- Control Test Mix
  - ToxBox (Screen)
    - 4-Fluoroisobutyryl fentanyl, 6-MAM, 7-aminoclonazepam, Acetyl fentanyl, Acrylfentanyl, Alprazolam, Amitriptyline, Amphetamine, Benzoylecgonine, Buprenorphine, Bupropion, Butyryl fentanyl, Carfentanyl, Carisoprodol, Citalopram, Clonazepam, Cocaine, Codeine, Cyclobenzaprine, Cyclopropyl fentanyl, Dextromethorphan, Diazepam, Dihydrocodeine, Diphenhydramine, Doxepin, Doxylamine, Duloxetine, Estazolam, Etizolam, Etomidate, Fentanyl, Flualprazolam, Flunitrazepam, Fluoxetine, Furanyl fentanyl, Hydrocodone, Hydromorphone, Ketamine, Levamisole, Loperamide, Lorazepam, MDA, MDMA, Meprobamate, Methadone, Methamphetamine, Midazolam, Mitragynine, Morphine, Nordiazepam, Norfentanyl, Norquetiapine, Nortriptyline, Orphenadrine, Oxazepam, Oxycodone, Oxymorphone, Phenazepam, Phencyclidine, Phentermine , Promethazine, Quetiapine, Sertraline,

Tapentadol, Temazepam, Tizanidine, Tramadol, Trazodone, Triazolam, Venlafaxine, Zolpidem, Zopiclone

- Internal Standard Test Mix
  - ToxBox (Screen)
    - 4-Fluoroisobutyryl fentanyl-D7, 6-MAM-D6, 7-Aminoclonazepam-D4. Acetyl fentanil-D5, Acrylfentanyl-D5, Alprazolam-D5, Amitriptyline-D3, Amphetamine-D11, Benzoylecgonine-D8, Buprenorphine-D4, Bupropion-D9, Butyryl fentanyl-D5, Carfentanyl-D5, Carisoprodol-D7, Citalopram-D6, Clonazepam-D4, Cocaine-D3, Codeine-D6, Cyclobenzaprine-D3,Cyclopropyl fentanyl-D5, Dextromethorphan-D3, Diazepam-D5, Dihydrocodeine-D6, Diphenhydramine-D3, Doxepin-D3, Doxylamine-D5, Duloxetine-D3, Estazolam-D5, Etizolam-D3, Fentanyl-D5, Flunitrazepam-D7, Fluoxetine-D6, Furanyl fentanyl-D5, Hydrocodone-D6, Hydromorphone-D6, Ketamine-D4, MDA-D5, MDMA-D5, Meprobamate-D7, Methadone-D9, Methamphetamine-D11, Midazolam-D4, Mitragynine-D3, Nordiazepam-D5, Norfentanyl-D5, Norquetiapine-D8, Nortriptyline-D3, Oxazepam-D5, Oxycodone-D6, Oxymorphone-D3, Phenazepam-D4, Phencyclidine-D5, Phentermine-D5, Promethazine-D3, Quetiapine-D8 ,Sertraline-D3, Tapentadol-D3, Temazepam-D5 ,Tizanidine-D4, Tramadol-13CD3, Trazodone-D6, Triazolam-D4, Venlafaxine-D6, Zolpidem-D6, Zopiclone-D4

Equipment

- Micropipettes and tips
- Benchtop vortex mixer
- Eppendorf 96-well plate shaker
- TurboVap 96-well plate concentration workstation
- SPE dry 96-well plate concentration workstation
- Eppendorf heat sealer
- 16x100 mm tubes

Instrumentation

- Agilent HPLC Agilent Triple quadrupole AJS source
- Kinetex™ 2.6 µm Phenyl-Hexyl 100 Å, LC Column 50×4.6 mm (Phenomenex part 00B-4495-E0, or equivalent)
- SecurityGuard™ ULTRA cartridges for Phenyl UHPLC (Phenomenex part AJ0-8774, or equivalent)

Instrument Conditions

Injection volume: 5 µL

Column oven: 35°C

Time (min)	Flow Rate (µL/min)	A%	B%
0	500	95	5
4.0	500	0	100
5.0	500	0	100
5.1	500	95	5
7.0	500	95	5

Compound Name	Precursor Ion (m/z)	Product ions (m/z)
4-Fluoroisobutyryl fentanyl	369.5	188.1
4-Fluoroisobutyryl fentanyl	369.5	105.1
4-Fluoroisobutyryl fentanyl-D7	376.5	188.1
6-MAM	328.4	211
6-MAM	328.4	165
6-MAM-D6	334.4	165
7-aminoclonazepam	286.1	222.1
7-aminoclonazepam	286.1	121.1
7-Aminoclonazepam-D4	290.1	121
Acetyl fentanil-D5	328.5	104.9
Acetyl fentanyl	323.5	188.1
Acetyl fentanyl	323.5	105.1
Acrylfentanyl	335.5	188.1
Acrylfentanyl	335.5	105.1
Acrylfentanyl-D5	340.5	105
Alprazolam	309	281.1
Alprazolam	309	206
Alprazolam-D5	314	286
Amitriptyline	278.4	105.1
Amitriptyline	278.4	91.1
Amitriptyline-D3	281.4	91
Amphetamine	136.2	119.1
Amphetamine	136.2	91.1
Amphetamine-D11	147.2	98.2
Benzoylecgonine	290.3	168
Benzoylecgonine	290.3	105
Benzoylecgonine-D8	298	171
Buprenorphine	468	396
Buprenorphine	468	55.3
Buprenorphine-D4	472	59.3
Bupropion	240	184
Bupropion	240	131
Bupropion-D9	249.2	185
Butyryl fentanyl	351.5	188.1

Butyryl fentanyl	351.5	105.1
Butyryl fentanyl-D5	356.5	188.1
Carfentanyl	395.5	113.1
Carfentanyl	395.5	105.1
Carfentanyl-D5	400.5	113.1
Carisoprodol	261.3	176.1
Carisoprodol	261.3	55.3
Carisoprodol-D7	268.4	183.1
Citalopram	325.4	109.1
Citalopram	325.4	83.1
Citalopram-D6	331.4	109.1
Clonazepam	316.1	270
Clonazepam	316.1	214
Clonazepam-D4	320.1	274.1
Cocaine	304.4	182.1
Cocaine	304.4	77.2
Cocaine-D3	307.4	185.1
Codeine	300.4	165
Codeine	300.4	152
Codeine-D6	306.4	152
Cyclobenzaprine	276.4	216.1
Cyclobenzaprine	276.4	215
Cyclobenzaprine-D3	279.4	215.1
Cyclopropyl fentanyl	349.4	188.1
Cyclopropyl fentanyl	349.4	105.1
Cyclopropyl fentanyl-D5	354.4	188.1
Dextromethorphan	272.4	171
Dextromethorphan	272.4	128
Dextromethorphan-D3	275.4	171
Diazepam	285.1	193
Diazepam	285.1	154
Diazepam-D5	290.1	198.1
Dihydrocodeine	302.4	199
Dihydrocodeine	302.4	128
Dihydrocodeine-D6	308.4	202.1
Diphenhydramine	256.4	167
Diphenhydramine	256.4	152
Diphenhydramine-D3	259.4	167.1
Doxepin	280.4	107.1
Doxepin	280.4	77.2
Doxepin-D3	283.4	107.1
Doxylamine	271.4	182.1
Doxylamine	271.4	167
Doxylamine-D5	276.4	187.1

Duloxetine	298.4	154
Duloxetine	298.4	44.3
Duloxetine-D3	301.4	47.3
Estazolam	295	267
Estazolam	295	206.1
Estazolam-D5	300	272
Etizolam	343	314
Etizolam	343	224
Etizolam-D3	346.9	318.1
Etomidate	245.3	141
Etomidate	245.3	95.1
Fentanyl	337.5	188
Fentanyl	337.5	105.1
Fentanyl-D5	342.5	188.1
Flualprazolam	327.8	293.1
Flualprazolam	327.8	224.1
Flunitrazepam	314.3	268.1
Flunitrazepam	314.3	239
Flunitrazepam-D7	321.3	275.1
Fluoxetine	310.3	148
Fluoxetine	310.3	44.3
Fluoxetine-D6	316.3	44.3
Furanyl fentanyl	375.5	188.1
Furanyl fentanyl	375.5	105.1
Furanyl fentanyl-D5	380.5	188.1
Hydrocodone	300.4	199
Hydrocodone	300.4	128.1
Hydrocodone-D6	306.4	202
Hydromorphone	286.4	185
Hydromorphone	286.4	157
Hydromorphone-D6	292.4	185
Ketamine	238	125
Ketamine-D4	242.7	130
Levamisole	205.3	178
Levamisole	205.3	91.1
Loperamide	478	267.2
Loperamide	478	266.7
Lorazepam	321	303
Lorazepam	321	229
MDA	180.2	163
MDA	180.2	105.1
MDA-D5	185.2	168
MDMA	194.2	163
MDMA	194.2	105.1

MDMA-D5	199.2	165
Meprobamate	219.3	158.1
Meprobamate	219.3	55.3
Meprobamate-D7	226.3	165.1
Methadone	310.5	265
Methadone	310.5	105.1
Methadone-D9	319.5	268.2
Methamphetamine	150.2	91.1
Methamphetamine	150.2	65.2
Methamphetamine-D11	161.2	97.2
Midazolam	326	291
Midazolam	326	249.1
Midazolam-D4	330	295.2
Mitragynine	399.5	174
Mitragynine	399.5	159
Mitragynine-D3	402.5	177.1
Morphine	286.4	201
Morphine	286.4	181
Morphine-D6	292.2	152
Nordiazepam	271.1	140
Nordiazepam	271.1	77.1
Nordiazepam-D5	276.1	140
Norfentanyl	233.3	84.2
Norfentanyl	233.3	55.3
Norfentanyl-D5	238.3	84.2
Norquetiapine	296.4	253
Norquetiapine	296.4	210
Norquetiapine-D8	304.4	210
Nortriptyline	264.4	105.1
Nortriptyline	264.4	91.1
Nortriptyline-D3	267.4	91
Orphenadrine	270.4	181
Orphenadrine	270.4	165
Oxazepam	287.1	269
Oxazepam	287.1	241
Oxazepam-D5	292.1	246
Oxycodone	316.4	298.1
Oxycodone	316.4	241.1
Oxycodone-D6	322.4	304.2
Oxymorphone	302.4	284.1
Oxymorphone	302.4	227.1
Oxymorphone-D3	305.4	287.2
Phenazepam	351	206
Phenazepam	351	179

Phenazepam-D4	353	183.9
Phencyclidine	244.4	91.1
Phencyclidine	244.4	86.2
Phencyclidine-D5	249.4	86.2
Phentermine	150.2	91.1
Phentermine	150.2	65.2
Phentermine-D5	155.2	96.1
Promethazine	285.4	86.2
Promethazine	285.4	71.2
Promethazine-D3	288.4	89.2
Quetiapine	384.5	253.1
Quetiapine	384.5	221
Quetiapine-D8	392.5	258.1
Sertraline	307.2	276.1
Sertraline	307.2	158.9
Sertraline-D3	310	158.9
Tapentadol	222.3	107.1
Tapentadol	222.3	77.2
Tapentadol-D3	225.3	107.1
Temazepam	301	255
Temazepam	301	177
Temazepam-D5	307	261
Tizanidine	254.7	45.2
Tizanidine	254.7	44.3
Tizanidine-D4	258	48.3
Tramadol	264.4	58.3
Tramadol	264.4	42.3
Tramadol-13C D3	268.4	58.3
Trazodone	372.9	176
Trazodone	372.9	148
Trazodone-D6	378.9	183.1
Triazolam	343	308
Triazolam	343	239
Triazolam-D4	347.2	312.1
Venlafaxine	278.4	260.2
Venlafaxine	278.4	58.3
Venlafaxine-D6	284.4	64.3
Zolpidem	308.4	235.1
Zolpidem	308.4	65.2
Zolpidem-D6	314.4	235.1
Zopiclone	389.1	245
Zopiclone	389.1	217
Zopiclone-D4	393.1	245

MS Acquisition Parameters:

MS Parameters	
Gas Temp (°C)	350
Gas Flow (l/min)	10
Nebulizer (psi)	45
Capillary Positive (V)	3500
Capillary Negative (V)	3500
MS1 Heater	100 °C
MS2 Heater	100 °C
Sheath Gas Temp (°C)	380
Sheath Gas flow (l/min)	11

Procedure

1. Turn on TurboVap and set at 35 °C. Turn on the shaking incubator and set at an ambient temperature.
2. Allow refrigerated specimens to reach ambient temperature.
3. Add 0.25 mL of blank blood to wells containing dried-down standards/QCs; add 0.25 mL of unknown sample to wells containing only internal standard. Mix several times via aspiration/dispensing.
4. Place the plate on the shaking incubator at approximately 900 rpm for approximately fifteen minutes.
5. Remove the plate and reset the temperature on the shaking incubator to an ambient temperature.
6. Add 0.25 mL of 0.5 M ammonium hydroxide to all wells. Mix several times via aspiration/dispensing.
7. Place plate on the shaking incubator at approximately 900 rpm for approximately fifteen minutes.
8. Transfer 300 µL of each well to the corresponding well of a SLE+ plate.
9. Apply vacuum until the solution penetrates the well sorbent approximately halfway (or until no liquid remains on top of sorbent). This should take approximately four seconds.
10. Wait five minutes for the sample to completely absorb.
11. Add 600 µL ethyl acetate and allow to flow for five minutes under gravity.
12. If necessary, apply vacuum to complete elution.
13. Repeat steps 11 & 12 twice for the second and third elution fractions.
14. Evaporate eluent plate to complete dryness in the TurboVap or SPE dry at approximately 35 °C under a constant flow of nitrogen (normally 10-15 units).
15. Reconstitute each sample with 100 µL methanol. Heat-seal the plate with foil to prevent evaporation. Place on shaker for approximately 5 minutes.
16. Analyze all samples immediately or store at approximately 4°.

Quality Assurance, Interpretation, Precautions, and Notes

This analytical technique does not produce a diagnostic mass spectrum (like that typical of EI GC-MS), so a different type of assessment is necessary to determine whether a targeted analyte is present. In order to report a "positive" result, the following criteria must be met:

Calibrators:
Up to four calibrators will be analyzed to set the linear range of the method. These calibrators will be prepared in a Tox Box. The concentrations of these calibrators will vary by analyte, with the expected working range listed in the validation document for each analyte. Calibrators outside of the validated working range may not be included in the assay.

The calibration curve should meet the requirements outlined in § 7.7.1, *Calibration Curves* excluding r-values of curves. The correlation coefficient of each analyte must be ≥ 0.85.

Controls:
The concentration of the positive control will vary by analyte, depending upon the expected working range of the assay for that analyte. Typically, one positive control concentration will be analyzed to evaluate the working range. The positive control must fall within ±50% of its target value and be within the working range of the calibration curve. If more than one positive control is extracted and analyzed due to the layout of the plate, the second positive control may be discarded without evaluation.

A negative control will be extracted (containing only internal standard) to demonstrate the absence of any contaminant which would result in a false positive response. The targeted analyte must not be detected (i.e., reportable) in the negative control.

Specimens:
One aliquot of each case specimen are typically run.

Signal-to-Noise Ratio (S:N):
The signal-to-noise ratio for each transition must be greater than 10:1. If no background noise exists for a given transition, then this requirement is considered to be met. Interfering peaks within the collection window may result in a lower signal-to-noise ratio for the analyte. Where there is an interfering peak, analytes with a S:N ≤ 10 may be reported if all other criteria are met (i.e., concentration of the analyte at or above the decision point, proper integration of the transition(s), and RRT). If the signal-to-noise ratio for the analyte is ≤ 5 due to an interfering peak, reporting that analyte requires the written approval of the Chief Forensic Toxicologist.

Retention Time:
The retention time must be within ±2% of the expected relative retention time, which is established by certified reference material. It is defined as the quotient of the retention time of the analyte and of the internal standard. The expected relative retention time is set in the instrument method, but may be evaluated on a batch-by-batch basis. An exception will be made for analytes that do not have an isotopically-labeled internal standard or specific analytes that are more suitable to a surrogate internal standard (e.g., Etomidate with Alprazolam-D5).

Other notes and requirements:
The qualitative presence of each analyte is determined by the evaluation the following parameters:

- Concentration of the analyte at or above the decision point
- Integration of transition(s)
- Relative retention time (RRT)
- Signal to noise (S:N)

This semi-quantitative analysis provides an estimated analyte concentration which will be used to interpret the presence or absence (not detected) of the analyte. This information may be used to direct the quantitative analysis of the analytes.

An analyte whose concentration is at or above the decision point will be reported as present. If a sample has a drug concentration below the analytes' decision, it will not be reported. This was administratively set for all analytes in the method validation.

Chromatographic peaks are automatically integrated during data analysis. It is the responsibility of the analyst to ensure that all appropriate chromatographic peaks are integrated. Analysts may sometimes need to adjust automatic integrations to properly represent the analytical data. Examples of these actions include: integrating the correct analyte among isobars, accounting for the qualifier ion for an analyte at a low concentration, and correcting an incomplete peak integration. A manual integration of apparent noise is not necessary to meet acceptability criteria to report an analyte in a sample or the associated case blank.

Sample plates may be stored for up to seven days after extraction, if stored refrigerated at 4 °C.

Solvent blanks or extracted blanks may be utilized to evaluate carryover.

Suspected carryover peaks for reportable analytes must be evaluated by the following:

Solvent blank
a) Less than 2% of the area of the peak for which it is blank, or
b) Less than 50% of the area of the lowest calibrator, or
c) Less than 1000 units within the detection time window

Extracted blank
a) The amount of analyte present in the blank cannot exceed the decision point
b) Elevated results (i.e., area of an analyte in the blank ≥ 50% of the area of the decision point) in blank injections following samples containing high amounts of analyte are evaluated on a case-by-case basis, and may require re-extraction or reanalysis before reporting

The performance of calibrators and controls is evaluated by a second analyst, and a record of this evaluation is recorded on a batch worksheet and maintained in the case record.

Preparation of Materials

LC Eluent "A"
0.631 grams of ammonium formate is added to a 1 L class A volumetric flask and brought to volume with ultrapure water. This solution expires after one month.

LC Eluent "B"
1 mL of formic acid is added to a 1 L class A volumetric flask and brought to volume with methanol. This solution expires after one month.

0.5 M Ammonium Hydroxide
33.57 mL of concentrated stock (28-30 % w/w) ammonium hydroxide is added to a 500 mL class A volumetric flask and brought to volume with ultrapure water. This solution expires after one month.

Needle Rinse Solution-
The composition of the needle rinse solution does not affect the quality of the data acquisition. The needle rinse is intended to reduce carryover. Suggested needle rinse solutions are listed below. Other variations of these solutions may be acceptable in casework. It is recommended to use HPLC grade solvents when applicable.

75% Methanol
750 mL of methanol is added to a 1L class A volumetric flask and brought to volume with ultrapure water. Isopropanol may be added to this solution as needed to help with carryover. This solution expires after six months.

60% Methanol, 20% Ultrapure Water, 20% Isopropanol
Combine 600 mL of methanol, 200 mL of ultrapure water, and 200 mL of isopropanol in an HPLC solvent bottle. This solution expires after six months.

0.1% Formic acid in 50% Methanol, 50% Ultrapure Water
Add 1 mL of concentrated formic acid to a 1:1 mixture of methanol and ultrapure water in an HPLC solvent bottle. This solution expires after six months.

95% LC Eluent "A" and 5% LC Eluent "B"
Combine 950 mL of LC Eluent "A" with 50 mL of LC Eluent "B" in an HPLC solvent bottle. This solution expires after one month.

9.13 LC-MS SMRM DRUG QUANTITATION

Scope

This method is designed to screen and/or quantitate members of a targeted list of analytes by tandem liquid chromatography-mass spectrometry scheduled multiple reaction monitoring (LC-MS sMRM) analysis.

This method is validated for use with blood specimens only. Calibrators, controls, and extracted blanks should be made in the appropriate matrix. A 500 or 600 µL aliquot is normally used, but other aliquot amounts may be used if appropriate.

This method is designed to detect the presence and/or quantitation of targeted analytes. The analytes are separated from their matrix by supported liquid extraction (SLE), separated from one another by HPLC, and detected by tandem mass spectrometry, monitoring two transitions for the analytes and one transition for isotopically-labeled internal standards.

Chemicals and Reagents

- Ultrapure water (17 MΩ-cm or greater)
- Ammonium formate
- LC-MS grade methanol (Fisher part A456-4)
- Ammonium hydroxide
- HPLC grade ethyl acetate
- Sheep blood
- SLE plates (Biotage part 820-0400-P01, or equivalent)
- 96-well plate Nunc (Fisher part 12-565-606, or equivalent)

Controls

- Tox Box (Quant) Agilent 6460C
    - Calibrators and controls: 6-Monoacetylmorphine, 7-Aminoclonazepam, Acetaminophen, Alprazolam, Amitriptyline, Amphetamine, Benzoylecgonine, Buprenorphine, Bupropion, Caffeine, Carisoprodol, Citalopram, Clonazepam, Cocaine, Codeine, Cyclobenzaprine, Dextromethorphan, Diazepam, Dihydrocodeine, Diphenhydramine, Fentanyl, Fluoxetine, Hydrocodone, Hydromorphone, Lorazepam, Methadone, Methamphetamine, Morphine, Nordiazepam, Oxycodone, Oxymorphone, Promethazine, Quetiapine, Sertraline, Tramadol, Trazodone, Venlafaxine, Zolpidem.

    - Internal Standard : 6-Monoacetylmorphine-D6, 7-Aminoclonazepam-D4, 7-Aminoflunitrazepam-D7, Acetaminophen-D4, Alprazolam-D5, Amitriptyline-D3, Amphetamine-D11, Benzoylecgonine-D8, Buprenorphine-D4, Bupropion-D9, Caffeine-^{13}C3, Carisoprodol-D7, Citalopram-D6, Clonazepam-D4, Cocaine-D3, Codeine-D6, Cyclobenzaprine-D3, Dextromethorphan-D3, Diazepam-D5,

Dihydrocodeine-D6, Diphenhydramine-D3, Fentanyl-D5, Fluoxetine-D6, Hydrocodone-D6, Hydromorphone-D6, Methadone-D9, Methamphetamine-D11, Morphine-D6, Nordiazepam-D5, Oxycodone-D6, Oxymorphone-D3, Promethazine-D3, Quetiapine-D8, Sertraline-D3, Tramadol-13C-D3, Trazodone-D6, Venlafaxine-D6, Zolpidem-D6.

Equipment

- Micropipettes and tips
- Benchtop vortex mixer
- Eppendorf 96-well plate shaker
- TurboVap 96-well plate concentration workstation
- SPE dry 96-well plate concentration workstation
- Eppendorf heat sealer
- 16×100 mm tubes

Instrumentation

- Agilent 6460C Series LC-MS Triple Quadrupole
  - "Hulk" ESI source
  - "Tony" and "Thor" AJS source
- Kinetex™ 2.6 µm Phenyl-Hexyl 100 Å, LC Column 50×4.6 mm (Phenomenex part 00B-4495-E0, or equivalent)
- SecurityGuard™ ULTRA cartridges for Phenyl UHPLC (Phenomenex part AJ0-8774, or equivalent)

Instrument Conditions

Agilent 6460C Instrument conditions
Injection volume: 5 µL
Column oven: 35°C

Time (min)	Flow Rate (µL/min)	A%	B%
0	500	95	5
4.0	500	0	100
5.0	500	0	100
5.1	500	95	5
7.0	500	95	5

Table 1 Hulk analytes and ions

Analyte	Precursor Ion (m/z)	Product Ions (m/z)
6-MAM	328.2	165
6-MAM	328.2	211
6-MAM-D6	334.2	165
7-aminoclonazepam	286.1	222.1
7-aminoclonazepam	286.1	121
7-Aminoclonazepam-D4	290.1	121
Acetaminophen	152.1	110
Acetaminophen	152.1	65.2
Acetaminophen-D4	156.1	114.1
Alprazolam	309.1	281.1
Alprazolam	309.1	205
Alprazolam-D5	314.1	286.1
Amitriptyline	278.2	105.1
Amitriptyline	278.2	91.1
Amitriptyline-D3	281.2	105.1
Amphetamine	136.1	119.1
Amphetamine	136.1	91.1
Amphetamine-D11	147.2	130.1
Benzoylecgonine	290.1	168
Benzoylecgonine	290.1	77.2
Benzoylecgonine-D8	298.2	171.1
Buprenorphine	468.3	396.2
Buprenorphine	468.3	55.2
Buprenorphine-D4	472.3	59.3
Bupropion	240.1	184
Bupropion	240.1	131
Bupropion-D9	249.2	185
Caffeine	195.1	138
Caffeine	195.1	42.3
Caffeine-^{13}C3	198.1	140
Carisoprodol	261.2	176.1
Carisoprodol	261.2	55.2
Carisoprodol-D7	268.2	183.1
Citalopram	325.2	262.1
Citalopram	325.2	109.1
Citalopram-D6	331.2	109
Clonazepam	316.1	270

Clonazepam	316.1	214
Clonazepam-D4	320.1	274.1
Cocaine	304.2	182.1
Cocaine	304.2	77.1
Cocaine-D3	307.2	185.1
Codeine	300.2	152
Codeine	300.2	115
Codeine-D6	306.2	152
Cyclobenzaprine	276.2	216.1
Cyclobenzaprine	276.2	215
Cyclobenzaprine-D3	279.2	215
Dextromethorphan	272.2	215.1
Dextromethorphan	272.2	171
Dextromethorphan-D3	275.2	171
Diazepam	285.1	193
Diazepam	285.1	154
Diazepam-D5	290.1	198.1
Dihydrocodeine	302.2	199
Dihydrocodeine	302.2	128
Dihydrocodeine-D6	308.2	202
Diphenhydramine	256.2	167
Diphenhydramine	256.2	165
Diphenhydramine-D3	259.2	167
Fentanyl	337.2	188.1
Fentanyl	337.2	105.1
Fentanyl-D5	342.3	105.1
Fluoxetine	310.1	148
Fluoxetine	310.1	44.3
Fluoxetine-D6	316.2	154.1
Hydrocodone	300.2	199
Hydrocodone	300.2	128
Hydrocodone-D6	306.2	202
Hydromorphone	286.2	185
Hydromorphone	286.2	157
Hydromorphone-D6	292.2	185
Lorazepam	321	303
Lorazepam	321	275
Methadone	310.2	265.1
Methadone	310.2	105
Methadone-D9	319.3	268.2

Methamphetamine	150.1	119.1
Methamphetamine	150.1	91.1
Methamphetamine-D11	161.2	97.1
Morphine	286.2	165
Morphine	286.2	152
Morphine-D6	292.2	152
Nordiazepam	271.1	165
Nordiazepam	271.1	140
Nordiazepam-D5	276.1	140
Nortriptyline	264.2	105.1
Nortriptyline	264.2	91.1
Nortriptyline-D3	267.2	105.1
Oxycodone	316.2	298.1
Oxycodone	316.2	241.1
Oxycodone-D6	322.2	304.2
Oxymorphone	302.1	284.1
Oxymorphone	302.1	227
Oxymorphone-D3	305.2	287.1
Promethazine	285.1	86.2
Promethazine	285.1	71.2
Promethazine-D3	288.2	89.2
Quetiapine	384.2	253.1
Quetiapine	384.2	221.1
Quetiapine-D8	392.2	226.1
Sertraline	306.1	275
Sertraline	306.1	158.9
Sertraline-D3	309.1	275
Tramadol	264.2	58.2
Tramadol	264.2	42.3
Tramadol-^{13}C-D3	268.2	58.2
Trazodone	372.2	176
Trazodone	372.2	148
Trazodone-D6	378.2	182.1
Venlafaxine	278.2	260.2
Venlafaxine	278.2	58.2
Venlafaxine-D6	284.2	64.3
Zolpidem	308.2	235.1
Zolpidem	308.2	92.1
Zolpidem-D6	314.2	235.1

Table 2 Tony analytes and ions

Compound Name	Precursor Ion (m/z)	Product ions (m/z)
6-MAM	328.2	211
6-MAM	328.2	165
6-MAM-D6	334.2	165
7-aminoclonazepam	286.1	222.1
7-aminoclonazepam	286.1	121.1
7-Aminoclonazepam-D4	290.1	121
Acetaminophen	152.1	110
Acetaminophen	152.1	65.2
Acetaminophen-D4	156.1	114.1
Alprazolam	309.1	281
Alprazolam	309.1	205.1
Alprazolam-D5	314.1	286
Amitriptyline	278.2	105.1
Amitriptyline	278.2	91.1
Amitriptyline-D3	281.2	91.1
Amphetamine	136.1	119.1
Amphetamine	136.1	91.1
Amphetamine-D11	147.2	98.1
Benzoylecgonine	290.1	168.1
Benzoylecgonine	290.1	77.1
Benzoylecgonine-D8	298.2	82.1
Buprenorphine	468.3	396
Buprenorphine	468.3	55.2
Buprenorphine-D4	472.3	59.3
Bupropion	240.1	184
Bupropion	240.1	131
Bupropion-D9	249.2	185
Caffeine	195.1	138
Caffeine	195.1	42.3
Caffeine-^{13}C3	198.1	140
Carisoprodol	261.2	176.1
Carisoprodol	261.2	55
Carisoprodol-D7	268.2	183.1
Citalopram	325.2	262
Citalopram	325.2	109.1
Citalopram-D6	331.2	109
Clonazepam	316.1	270
Clonazepam	316.1	214
Clonazepam-D4	320.1	274.1
Cocaine	304.2	182.1
Cocaine	304.2	77.1

Cocaine-D3	307.2	185.1
Codeine	300.2	152.1
Codeine	300.2	115
Codeine-D6	306.2	152
Cyclobenzaprine	276.2	216.1
Cyclobenzaprine	276.2	215
Cyclobenzaprine-D3	279.2	215.1
Dextromethorphan	272.2	215.1
Dextromethorphan	272.2	171.1
Dextromethorphan-D3	275.2	171.1
Diazepam	285.1	193
Diazepam	285.1	154
Diazepam-D5	290.1	198.1
Dihydrocodeine	302.2	199
Dihydrocodeine	302.2	128
Dihydrocodeine-D6	308.2	202.1
Diphenhydramine	256.2	167.1
Diphenhydramine	256.2	165.1
Diphenhydramine-D3	259.2	167.1
Fentanyl	337.2	188.1
Fentanyl	337.2	105.1
Fentanyl-D5	342.3	105.1
Fluoxetine	310.1	148.1
Fluoxetine	310.1	44.2
Fluoxetine-D6	316.2	154
Hydrocodone	300.2	199
Hydrocodone	300.2	128
Hydrocodone-D6	306.2	202.1
Hydromorphone	286.2	185.1
Hydromorphone	286.2	157
Hydromorphone-D6	292.2	185
Lorazepam	321	303
Lorazepam	321	275
Lorazepam-D4	325.1	307
Methadone	310.2	265.1
Methadone	310.2	105.1
Methadone-D9	319.3	268
Methamphetamine	150.1	119.1
Methamphetamine	150.1	91.1
Methamphetamine-D11	161.2	97.1
Morphine	286.2	165.1
Morphine	286.2	152.1
Morphine-D6	292.2	152
Nordiazepam	271.1	140

Nordiazepam	271.1	77.1
Nordiazepam-D5	276.1	140
Nortriptyline	264.2	105.1
Nortriptyline	264.2	91.1
Nortriptyline-D3	267.2	91.1
Oxycodone	316.2	298.1
Oxycodone	316.2	241.1
Oxycodone-D6	322.2	304.1
Oxymorphone	302.1	284.1
Oxymorphone	302.1	227.1
Oxymorphone-D3	305.2	287.1
Promethazine	285.1	86.2
Promethazine	285.1	71.2
Promethazine-D3	288.2	89.2
Quetiapine	384.2	253
Quetiapine	384.2	221.1
Quetiapine-D8	392.2	226
Sertraline	306.1	275
Sertraline	306.1	159
Sertraline-D3	309.1	159
Tramadol	264.2	58.2
Tramadol	264.2	42.2
Tramadol-13C-D3	268.2	58.2
Trazodone	372.2	176.1
Trazodone	372.2	148.1
Trazodone-D6	378.2	182.1
Venlafaxine	278.2	260.2
Venlafaxine	278.2	58.2
Venlafaxine-D6	284.2	64.2
Zolpidem	308.2	235.1
Zolpidem	308.2	65.2
Zolpidem-D6	314.2	235.1

MS Acquisition Parameters “Hulk”:

Acquisition method	Quant Method
Gas Temp	350° C
Gas Flow	12 L/min
Nebulizer	50
Capillary positive	4000 V
Capillary negative	4000 V
MS1 Heater	100° C
MS2 Heater	100° C

MS Acquisition Parameters “Tony”:

Acquisition method	Quant Method
Gas Temp	350° C
Gas Flow	5 L/min
Nebulizer	35
Capillary positive	3500 V
Capillary negative	3500 V
MS1 Heater	100° C
MS2 Heater	100° C
Sheath Gas Temp	380° C
Sheath Gas Flow	11 L/min

Procedure

1. Turn on TurboVap and set at 35 °C. Turn on the shaking incubator and set at an ambient temperature.
2. Allow refrigerated specimens to reach ambient temperature.
3. Add 0.25 mL of blank blood to wells containing dried-down standards/QCs; add 0.25 mL of unknown sample to wells containing only internal standard. Mix several times via aspiration/dispensing.
4. Place the plate on the shaking incubator at approximately 900 rpm for approximately fifteen minutes.
5. Remove the plate and reset the temperature on the shaking incubator to an ambient temperature.
6. Add 0.25 mL of 0.5 M ammonium hydroxide to all wells. Mix several times via aspiration/dispensing.
7. Place plate on the shaking incubator at approximately 900 rpm for approximately fifteen minutes.
8. Transfer 300 µL of each well to the corresponding well of a SLE+ plate.
9. Apply vacuum until the solution penetrates the well sorbent approximately halfway (or until no liquid remains on top of sorbent). This should take approximately four seconds.
10. Wait five minutes for the sample to completely absorb.

11. Add 600 µL ethyl acetate and allow to flow for five minutes under gravity.
12. If necessary, apply vacuum to complete elution.
13. Repeat steps 11 & 12 twice for the second and third elution fraction.
14. Evaporate eluent plate to complete dryness in the TurboVap or SPE dry at approximately 35 °C under a constant flow of nitrogen (normally 10-15 units).
15. Reconstitute each sample with 100 µL methanol. Heat-seal the plate with foil to prevent evaporation. Place on shaker for approximately 5 minutes.
16. Analyze all samples immediately or store at approximately 4°C.

Quality Assurance, Interpretation, Precautions, and Notes

This analytical technique does not produce a diagnostic mass spectrum (like that typical of EI GC-MS), so a different type of assessment is necessary to determine whether a targeted analyte is present. In order to report a "positive" result, the following criteria must be met:

Calibrators:
Up to seven calibrators will be analyzed to set the linear range of the method. These calibrators will be prepared in a Tox Box. The concentrations of these calibrators will vary by analyte, with the expected working range listed in the validation document for each analyte. Calibrators outside of the validated working range may not be included in the assay.

The calibration curve must meet the requirements outlined in § 7.7.1, *Calibration Curves*.

Controls:
The concentration of the positive controls will vary by analyte, depending upon the expected working range of the assay for that analyte. Typically, four positive control concentrations will be analyzed to evaluate the working range. The CRM used to prepare the positive control must be from a different source than the CRM used to prepare the calibrators. Each positive control must fall within 20% of its target value (30% for the concentration of the lowest calibrator) and be within the working range of the calibration curve.

A negative control will be extracted (containing only internal standard) to demonstrate the absence of any contaminant which would result in a false positive response. The targeted analyte must not be detected (i.e., reportable) in the negative control.

Specimens:
Two aliquots of each case specimen are typically run. The quantitative results of any two aliquots of the same specimen may not deviate more than 20% from their mean.

Dilutions of the case specimen may include a 1:10 dilution depending on the analyte and the method. Dilutions are made using sheep blood—no other diluent may be used to dilute the specimen.

Care should be taken to ensure integration of all analyte peaks (qualifier ion) at the correct RRT within the sample, when analytes in reportable amounts are observed for diluted and undiluted specimens.

Signal-to-Noise Ratio (S:N):
The signal-to-noise ratio for each transition must be greater than 10:1. If no background noise exists for a given transition, then this requirement is considered to be met. Interfering peaks within the collection window may result in a lower signal-to-noise ratio for the analyte. Where there is an interfering peak, analytes with a S:N ≤ 10 may be reported if all other criteria are met (i.e., concentration of the analyte at or above the decision point, proper integration of the transition(s), and RRT). If the signal-to-noise ratio for the analyte is ≤ 5 due to an interfering peak, reporting that analyte requires the written approval of the Chief Forensic Toxicologist.

Retention Time:
The relative retention time (RRT) of the analyte and the internal standard shall be established using corresponding certified reference materials. The estimated RRT for each analyte shall be within 0.02 of the expected RRT assessed during analysis. Relative retention time is defined as the quotient of the retention time of the analyte and of the internal standard. The expected relative retention time is set in the instrument method, but may be evaluated on a batch-by-batch basis.

Other notes and requirements:
The qualitative presence of each analyte is determined by the evaluation and integration of the Q1 and Q2 transitions, the signal to noise (S:N), and the relative retention time (RRT) for each analyte.

If a case undergoes semi-quantitative screen analysis (§9.13), then a truncated evaluation of the quantitative data may be performed in those cases. Only those analytes reported as present during the semi-quantitative analysis may be evaluated for quantitative values. Exclusions may include acetaminophen and caffeine but all analytes can be evaluated on a case-by-case basis.

Chromatographic peaks are automatically integrated during data analysis. It is the responsibility of the analyst to ensure that all appropriate chromatographic peaks are integrated. Analysts may sometimes need to adjust automatic integrations to properly represent the analytical data. Examples of these actions include: integrating the correct analyte among isobars, accounting for the qualifier ion for an analyte at a low concentration, and correcting an incomplete peak integration. A manual integration of apparent noise is not necessary to meet acceptability criteria to report an analyte in a sample or the associated case blank.

Sample plates may be stored for up to seven days after extraction, if stored refrigerated at 4 °C.

Solvent blanks or extracted blanks may be utilized to evaluate carryover.

Suspected carryover peaks for reportable analytes must be evaluated by the following:

Solvent blank

a) Less than 2% of the area of the peak for which it is blank, or

b) Less than 50% of the area of the lowest calibrator, or
c) Less than 1000 units within the detection time window

Extracted blank

a) Concentration of the analyte shall not be detected within the set curve range for that drug (e.g., 25 ng/mL to 1000 ng/mL for methamphetamine)
b) The integrated analyte peak integration must be within the retention time window

The performance of calibrators and controls is evaluated by a second analyst, and a record of this evaluation is recorded on a batch worksheet and maintained in the case record.

If a sample has a drug concentration below the curve's limit of detection, it is not typically reported. Exceptions may be made by the Chief Forensic Toxicologist based on the data and the needs of the case.

Preparation of Materials

LC Eluent "A"
0.631 grams of ammonium formate is added to a 1 L class A volumetric flask, and brought to volume with ultrapure water. This solution expires after one month.

LC Eluent "B"
1 mL of formic acid is added to a 1 L class A volumetric flask and brought to volume with methanol. This solution expires after one month.

0.5 M Ammonium Hydroxide
33.57 mL of concentrated stock (28-30 % w/w) ammonium hydroxide is added to a 500 mL class A volumetric flask and brought to volume with ultrapure water. This solution expires after one month.

Needle Rinse Solution-
The composition of the needle rinse solution does not affect the quality of the data acquisition. The needle rinse is intended to reduce carryover. Suggested needle rinse solutions are listed below. Other variations of these solutions may be acceptable in casework. It is recommended to use HPLC grade solvents when applicable.

75% Methanol
750 mL of methanol is added to a 1L class A volumetric flask and brought to volume with ultrapure water. Isopropanol may be added to this solution as needed to help with carryover. This solution expires after six months.

60% Methanol, 20% Ultrapure Water, 20% Isopropanol
Combine 600 mL of methanol, 200 mL of ultrapure water, and 200 mL of isopropanol in an HPLC solvent bottle. This solution expires after six months.

0.1% Formic acid in 50% Methanol, 50% Ultrapure Water
Add 1 mL of concentrated formic acid to a 1:1 mixture of methanol and ultrapure water in an HPLC solvent bottle. This solution expires after six months.

95% LC Eluent "A" and 5% LC Eluent "B"
Combine 950 mL of LC Eluent "A" with 50 mL of LC Eluent "B" in an HPLC solvent bottle. This solution expires after one month.

9.14 LC-MS SMRM DRUG QUANTITATION THC IN BLOOD

Scope

This method is designed to screen and quantitate Δ-9-Tetrahydrocannabinol (THC) and its metabolites by tandem liquid chromatography-mass spectrometry scheduled multiple reaction monitoring (LC-MS sMRM) analysis.

This method is validated for use with blood specimens only. Calibrators, controls, and extracted blanks should be made in the appropriate matrix. Two 1 mL aliquots are normally used, but other aliquot amounts may be used if appropriate.

This method is designed to detect the presence and quantitation of targeted analytes. The analytes are separated from their matrix by supported liquid extraction (SLE), separated from one another by HPLC, and detected by tandem mass spectrometry, monitoring two transitions for the analytes and one transition for isotopically-labeled internal standards.

Chemicals and Reagents

- Ultrapure water (17 MΩ-cm or greater)
- LC-MS grade formic acid (Fisher part A117-50, or equivalent)
- Ammonium formate
- LC-MS grade methanol (Fisher part A456-4)
- Methyl tert-butyl ether, 99.9% grade (Acros Organics part 378720025, or equivalent)
- HPLC grade acetonitrile (VWR part EM-AX0145P-1, or equivalent)
- LC-MS grade Hexanes (Fisher part H303-4, or equivalent)
- ACS grade DMSO (Fisher part D128-1, or equivalent)
- Sheep blood
- Isolute SLE + 1 mL SLE plates (Biotage part 820-1000-Q01, or equivalent)
- 48-well plate (Artic White part AWLS-360002, or equivalent)

Controls

- Tox Box (THC)
    - Calibrators and controls: Δ^9-Tetrahydrocannabinol (THC), 11-Nor-9-carboxy-THC (THC-COOH), 11-Hydroxy-Δ^9-tetrahydrocannabinol (THC-OH)
    - Internal Standard : THC-D3, THC-COOH-D9, THC-OH-D3

Equipment

- Micropipettes and tips
- Benchtop vortex mixer
- Eppendorf 96-well plate shaker
- TurboVap 96-well plate concentration workstation
- TurboVap LV concentration workstation
- Eppendorf heat sealer
- 16×100 mm tubes

Instrumentation

- AB SCIEX 4000 Q TRAP LC/MS/MS system
- Selectra DA 100 x 2.1 mm, 3 µm (UCT part SLDA100ID21-3UM, or equivalent)

Instrument Conditions

Injection volume: 10 µL
Column oven: 50 °C

Time (min)	Flow Rate (µL/min)	A%	B%
0	600	45	55
3.5	600	45	55
3.6	600	20	80
5.5	600	20	80
5.6	600	45	55
8.0	600	45	55

Analyte	Range (ng/mL)	LOD	LOQ	Parent	Quant	Qual
THC	1-100	1	1	315.2	193.1	123.1
THC-D3	-	-	-	318.2	196.1	-
THC-COOH	5-250	5	5	345.2	327.2	299.1
THC-COOH-D9	-	-	-	354.2	336.2	-
THC-OH	5-100	5	5	331.2	313.2	193.0
THC-OH-D3	-	-	-	334.2	316.1	-

Acquisition method	THC Quant Method
Sync mode	LC Sync
Auto-equilibration	Off
Acquisition time	8.000 min
Number of periods	1
Scans per period	960
Scan type	sMRM
Ion source	Turbospray
MRM detection window	240 sec
Target scan time	0.5 sec
Resolution (Q1)	Unit
Resolution (Q3)	Unit
Intensity threshold	0
Settling time	0
MR pause	5.007 ms
MCA	No
Step size	0.00 Da

Procedure

1. Remove standards plate, blood, and samples from cold storage. Allow to reach room temperature.
2. Pipette 1 mL (1000 µL) blood in wells of analytical (standards) plate.
   - Blank blood for locations containing standards/QCs and internal standards and extracted blank.
   - Sample blood for locations containing only internal standards. Mix several times via aspiration/dispensing.
3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
4. Add 500 µL of 0.1% formic acid (THC mobile phase A) to all wells. Mix several times via aspiration/dispensing.
5. Place on shaking incubator at ambient temperature, 900rpm for 15 minutes.
6. Transfer 400 µL of mixture to corresponding wells of 48 well-SLE+ plate
7. Apply positive pressure for approx. 4 seconds (or until no liquid remains on top of sorbent). Wait 5 min.
8. Add 2.25 mL MTBE. Wait for few minutes between aliquots to allow flow under gravity. If necessary, apply positive pressure between aliquots. After the transfer wait for five minutes to allow the flow under gravity.
9. Apply positive pressure for approx. 15 seconds.
10. Add 2.25 mL hexane, and follow the directions in Step. 8.
11. Apply positive pressure for approx. 15 seconds.
12. Remove plate. Place on TurboVap and evaporate to dryness at 35°C with a flow rate of 10-12. After 15 minutes, check the plate and increase flow rate to 16-20. Continue monitoring every 15 minutes.
13. Reconstitute the dry plate in 100 µL methanol and heat seal plate with foil. Shake at ambient temp, 900 rpm for 5 minutes.

INSTRUMENTATION

1. Check the needle wash solvent container, fill if necessary (75% v/v methanol in water). Empty the condensate-waste bottle, & the solvents waste.
2. Attach new mobile phases to the LC. Set the LC to pump to waste by turning the purge valve (black knob) gently to the left. Adjust the flow rate to about 2.0 mL/min and a 50:50 gradient (Do not increase the flow rate above 1ml/min unless the valve is open). Push CTRL, 1, on. Let flow ~ 15 minutes. Ensure all bubbles are out of the line.
3. Decrease flow rate to 0.6 mL/min and set A to 45%. Remove tubing going into mass spec and place in waste container. Gently close the knob by turning it to the right. Ensure mobile phase is flowing through tubing. Allow to flow for few minutes. The backpressure percentage should be around 0%.
4. Reattach the tubing to the mass spec. The pressure will increase. Wait until the pressure has stabilized before starting the sequence.
5. Prime pump using the same module (press control, select option 5). Clean the needle for at least 1 minute using the hand-held command module (press control, select option 4). Allow the needle to reposition after the wash.
6. Using analyst software begin the equilibration (usually 1 min using the method that is automatically selected). After the successful equilibration all the icons on the bottom right-hand corner will turn green. At this time the sequence can be started for data collection.

Quality Assurance, Interpretation, Precautions, and Notes

This analytical technique does not produce a diagnostic mass spectrum (like that typical of EI GC-MS), so a different type of assessment is necessary to determine whether a targeted analyte is present. In order to report a "positive" result, the following criteria must be met:

Calibrators:
Up to eight calibrators will be analyzed to set the linear range of the method. These calibrators will be prepared in a Tox Box. The concentrations of these calibrators will vary by analyte, with the expected working range listed in the validation document for each analyte. Calibrators outside of the validated working range may not be included in the assay.

The calibration curve must meet the requirements outlined in § 7.7.1, *Calibration Curves*.

Controls:
Typically, four positive control concentrations will be analyzed to evaluate the working range. The CRM used to prepare the positive control must be from a different source than the CRM used to prepare the calibrators. Each positive control must fall within 20% of its target value (30% for the concentration of the lowest calibrator) and be within the working range of the calibration curve.

A negative control will be extracted (containing only internal standard) to demonstrate the absence of any contaminant which would result in a false positive response. The targeted analyte must not be detected (i.e., reportable) in the negative control.

Specimens:
Typically one aliquot of each case specimen are run for qualitative testing while two aliquots of each case specimen are run for quantitative analysis, if sample amount permits. The quantitative results of the two aliquots of the same specimen may not deviate more than 20% from their mean. Dilutions of the case specimen may include a 1:10 dilution. Dilutions are made using sheep blood—no other diluent may be used to dilute the specimen.

Signal-to-Noise Ratio (S:N):
The signal-to-noise ratio for each transition must be greater than 10:1. If no background noise exists for a given transition, then this requirement is considered to be met. Interfering peaks within the collection window may result in a lower signal-to-noise ratio for the analyte. Where there is an interfering peak, analytes with a S:N ≤ 10 may be reported if all other criteria are met (i.e., concentration of the analyte at or above the decision point, proper integration of the transition(s), and RRT). If the signal-to-noise ratio for the analyte is ≤ 5 due to an interfering peak, reporting that analyte requires the written approval of the Chief Forensic Toxicologist.

Retention Time:
The retention time must be within ±4% of the expected relative retention time, which is established by certified reference material. It is defined as the quotient of the retention time of the analyte and

of the internal standard. The expected relative retention time is set in the instrument method, but may be evaluated on a batch-by-batch basis.

Other notes and requirements:
The qualitative presence of each analyte is determined by the evaluation and integration of the Q1 and Q2 transitions, the signal to noise (S:N), and the relative retention time (RRT) for each analyte.

Chromatographic peaks are automatically integrated during data analysis. It is the responsibility of the analyst to ensure that all appropriate chromatographic peaks are integrated. Analysts may sometimes need to adjust automatic integrations to properly represent the analytical data. Examples of these actions include: integrating the correct analyte among isobars, accounting for the qualifier ion for an analyte at a low concentration, and correcting an incomplete peak integration. A manual integration of apparent noise is not necessary to meet acceptability criteria to report an analyte in a sample or the associated case blank.

Sample plates may be stored for up to seven days after extraction, if stored refrigerated at 4 °C. Carryover is monitored by running a solvent blank between case specimens. Carryover may be evaluated by calculating 2% of the area of the peak for which it is blank or with 50% of the area of the lowest calibrator for each analyte. Any solvent blank carrying a chromatographic peak of an intensity less than 1000 units or less within the detection time window, is deemed acceptable in casework. Suspected carryover peaks greater than 1000, will be evaluated on a case by case basis.

Positive results in injections following samples containing high amounts of analyte are evaluated on a case-by-case basis, and may require re-extraction or reanalysis before reporting.

The performance of calibrators and controls is evaluated by a second analyst, and a record of this evaluation is recorded on a batch worksheet and maintained in the case record.

If a sample has a drug concentration below the curve's limit of detection, it may not be reported. This was administratively set for all analytes in the method validation.

Preparation of Materials

LC Eluent "A"
1 mL of formic acid is added to a 1 L class A volumetric flask, and brought to volume with ultrapure water. This solution expires after one month.

LC Eluent "B"
1 mL of formic acid is added to a 1 L class A volumetric flask and brought to volume with acetonitrile. This solution expires after one month.

0.1% Aqueous Formic Acid
1 mL of formic acid is added to a 1 L class A volumetric flask and brought to volume with ultrapure water. This solution expires after one month.

Needle Rinse Solution-

The composition of the needle rinse solution does not affect the quality of the data acquisition. The needle rinse is intended to reduce carryover. Suggested needle rinse solutions are listed below. Other variations of these solutions may be acceptable in casework. It is recommended to use HPLC grade solvents when applicable.

75% Methanol
750 mL of methanol is added to a 1L class A volumetric flask and brought to volume with ultrapure water. Isopropanol may be added to this solution as needed to help with carryover. This solution expires after six months.

60% Methanol, 20% Ultrapure Water, 20% Isopropanol
Combine 600 mL of methanol, 200 mL of ultrapure water, and 200 mL of isopropanol in an HPLC solvent bottle. This solution expires after six months.

0.1% Formic acid in 50% Methanol, 50% Ultrapure Water
Add 1 mL of concentrated formic acid to a 1:1 mixture of methanol and ultrapure water in an HPLC solvent bottle. This solution expires after six months.

95% LC Eluent "A" and 5% LC Eluent "B"
Combine 950 mL of LC Eluent "A" with 50 mL of LC Eluent "B" in an HPLC solvent bottle. This solution expires after one month.

9.15 LC-MS SMRM DRUG QUANTITATION THC IN URINE

Scope

This method is designed to screen and quantitate Δ-9-Tetrahydrocannabinol (THC) and its metabolites by tandem liquid chromatography-mass spectrometry scheduled multiple reaction monitoring (LC-MS sMRM) analysis.

This method is validated for use with urine specimens only. Calibrators, controls, and extracted blanks should be made in the appropriate matrix. One 250 µL aliquot is normally used.

This method is designed to detect the presence and quantitation of targeted analytes. The analytes are separated from their matrix by supported liquid extraction (SLE), separated from one another by HPLC, and detected by tandem mass spectrometry, monitoring two transitions for the analytes and one transition for isotopically-labeled internal standards.

Chemicals and Reagents

- Ultrapure water (17 MΩ-cm or greater)
- LC-MS grade formic acid (Fisher part A117-50, or equivalent)
- LC-MS grade methanol (Fisher part A456-4)
- Methyl tert-butyl ether, 99.9% grade (Acros Organics part 378720025, or equivalent)
- HPLC grade acetonitrile (VWR part EM-AX0145P-1, or equivalent)
- HPLC grade Hexanes
- Blank urine
- SLE plates (Biotage part 60109-500-2-9W, or equivalent)
- 96-well plate Nunc (Fisher part 12-565-606, or equivalent)

Controls

- Tox Box (THC)
    - Calibrators and controls: Δ^9-Tetrahydrocannabinol (THC), 11-Nor-9-carboxy-THC (THC-COOH), 11-Hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), Cannabidiol
    - Internal Standard : THC-D3, THC-COOH-D9, THC-OH-D3, Cannabidiol-D9
    - Deconjugation plate (PinPoint part GLC-701, or equivalent)

Equipment

- Micropipettes and tips
- Benchtop vortex mixer
- Eppendorf 96-well plate shaker
- TurboVap 96-well plate concentration workstation
- TurboVap LV concentration workstation
- Eppendorf heat sealer

Instrumentation

- Agilent 6460C LC/MS/MS system
- Selectra DA 100 x 2.1 mm, 3 µm (UCT part SLDA100ID21-3UM, or equivalent)

Instrument Conditions

Injection volume: 10 µL
Column oven: 50 °C

Time (min)	Flow Rate (µL/min)	A%	B%
0	600	45	55
3.5	600	45	55
3.6	600	20	80
5.5	600	20	80
Post run (2.5)	600	45	55

Analyte	Range (ng/mL)	LOD	LOQ	Parent	Quant	Qual
THC	10-250	10	10	315.2	193.1	123.1
THC-D3	-	-	-	318.2	196.1	-
THC-COOH	10-250	10	10	345.2	327.2	299.1
THC-COOH-D9	-	-	-	354.2	336.2	-
THC-OH	10-250	10	10	331.2	313.2	43.1
THC-OH-D3	-	-	-	334.3	316.2	-
Cannabidol	10-250	10	10	315.2	193.1	123.0
Cannabidiol-D9	-	-	-	324.3	202.1	-

Parameter	Setting
Column Type	UCT Selectra DA 100 x 2.1mm, 3um
Mass spectrometer mode	Positive electrospray ionization, dynamic multiple reaction monitoring (sMRM)
Injection Volume	10 µl
Column Heater	50 ºC
Gas Temperature	350 ºC
Gas Flow	5 L/min [Nitrogen]
Nebulizer Gas	35 psi [Nitrogen]
Sheath Gas Temperature	380 ºC
Sheath Gas Flow	11 L/min [Nitrogen]
Capillary Voltage	3500 V
Cell Accelerator Voltage	4 V

Procedure

1. Remove standards plate, urine, and samples from cold storage. Allow to reach room temperature.
2. Pipette 250 µL in wells of analytical (standards) plate.
   - Blank urine for locations containing standards/QCs and internal standards and extracted blank.

- Sample urine for locations containing only internal standards. Mix several times via aspiration/dispensing.

3. Transfer contents of deconjugation plate, 250 µL, to corresponding wells of the analytical plate.
4. Place plate on the shaking incubator for 3 hours (900 rpm at 60°C).
5. Transfer 300 µL of the urine+enzyme mixture to corresponding wells of 96-well SLE+ plate.
6. Apply positive pressure for approx. 4 seconds (or until no liquid remains on top of sorbent). Wait 5 min.
7. Add 900 µL MTBE and allow to flow for 5 minutes under gravity.
8. Apply positive pressure for approx. 15 seconds.
9. Add 900 µL hexane and allow to flow for 5 minutes under gravity.
10. Apply positive pressure for approx. 15 seconds.
11. Remove plate. Place on TurboVap and evaporate to dryness at 35°C with a flow rate of 10-12. After 15 minutes, check the plate and increase flow rate to 16-20. Continue monitoring every 15 minutes.
12. Reconstitute the dry plate in 100 µL methanol and heat seal plate with foil. Shake at ambient temp, 900rpm for 5 minutes.

INSTRUMENTATION

1. Check the reservoirs on the HPLC to ensure sufficient mobile phase is present.
2. Check the needle rinse solvent level to ensure there is plenty of solvent to perform the run.
3. The instrument should be equilibrated with fresh mobile phase before the run.
4. A solvent blank should be injected first to ensure the baseline is clean.
5. A check standard should be injected to allow for chromatography and instrument performance assessment.

Quality Assurance, Interpretation, Precautions, and Notes

This analytical technique does not produce a diagnostic mass spectrum (like that typical of EI GC-MS), so a different type of assessment is necessary to determine whether a targeted analyte is present. In order to report a "positive" result, the following criteria must be met:

Calibrators:
Up to eight calibrators will be analyzed to set the linear range of the method. These calibrators will be prepared onto a Tox Box. The concentrations of these calibrators will vary by analyte, with the expected working range listed in the validation document for each analyte. Calibrators outside of the validated working range may not be included in the assay.

The calibration curve should meet the requirements outlined in § 7.7.1, *Calibration Curves* excluding r-values of curves.

Controls:
Typically, four positive control concentrations will be analyzed to evaluate the working range. The CRM used to prepare the positive control must be from a different source than the CRM used to prepare the calibrators.

Each positive control must fall within 20% of its target value (30% for the concentration of the lowest calibrator) and be within the working range of the calibration curve.

A negative control will be extracted (containing only internal standard) to demonstrate the absence of any contaminant which would result in a false positive response. The targeted analyte must not be detected (i.e., reportable) in the negative control.

Specimens:
One aliquot of each case specimen are typically run.

Signal-to-Noise Ratio (S:N):
The signal-to-noise ratio for each transition must be greater than 8:1. If no background noise exists for a given transition, then this requirement is considered to be met. Interfering peaks within the collection window may result in a lower signal-to-noise ratio for the analyte. Where there is an interfering peak, analytes with a S:N ≤ 8 may be reported if all other criteria are met (i.e., concentration of the analyte at or above the decision point, proper integration of the transition(s), and RRT). If the signal-to-noise ratio for the analyte is ≤ 5 due to an interfering peak, reporting that analyte requires the written approval of the Chief Forensic Toxicologist.

Retention Time:
The retention time must be within ±4% of the expected relative retention time, which is established by certified reference material. It is defined as the quotient of the retention time of the analyte and of the internal standard. The expected relative retention time is set in the instrument method, but may be evaluated on a batch-by-batch basis.

Other notes and requirements:
The qualitative presence of each analyte is determined by the evaluation of the Q1 and Q2 transitions, the signal to noise (S:N), and the relative retention time (RRT) for each analyte.

Chromatographic peaks are automatically integrated during data analysis. It is the responsibility of the analyst to ensure that all appropriate chromatographic peaks are integrated. Analysts may sometimes need to adjust automatic integrations to properly represent the analytical data. Examples of these actions include: integrating the correct analyte among isobars, accounting for the qualifier ion for an analyte at a low concentration, and correcting an incomplete peak integration. A manual integration of apparent noise is not necessary to meet acceptability criteria to report an analyte in a sample or the associated case blank.

Sample plates may be stored for up to seven days after extraction, if stored refrigerated at 4 °C. Carryover is monitored by running a solvent blank between case specimens. Carryover may be evaluated by calculating 2% of the area of the peak for which it is blank or with 50% of the area of the lowest calibrator for each analyte. Any solvent blank carrying a chromatographic peak of an intensity less than 1000 units or less within the detection time window, is deemed acceptable in casework. Suspected carryover peaks greater than 1000, will be evaluated on a case by case basis.

Positive results in injections following samples containing high amounts of analyte are evaluated on a case-by-case basis, and may require re-extraction or reanalysis before reporting.

The performance of calibrators and controls is evaluated by a second analyst, and a record of this evaluation is recorded on a batch worksheet and maintained in the case record.

If a sample has a drug concentration below the curve's limit of detection, it may not be reported. This was administratively set for all analytes in the method validation.

Preparation of Materials

LC Eluent "A" 0.1% Formic Acid in Water
1 mL of formic acid is added to a 1 L class A volumetric flask, and brought to volume with ultrapure water. This solution expires after one month.

LC Eluent "B" 0.1% Formic Acid in Acetonitrile
1 mL of formic acid is added to a 1 L class A volumetric flask and brought to volume with acetonitrile. This solution expires after one month.

Needle Rinse Solution-
The composition of the needle rinse solution does not affect the quality of the data acquisition. The needle rinse is intended to reduce carryover. Suggested needle rinse solutions are listed below. Other variations of these solutions may be acceptable in casework. It is recommended to use HPLC grade solvents when applicable.

75% Methanol
750 mL of methanol is added to a 1L class A volumetric flask and brought to volume with ultrapure water. Isopropanol may be added to this solution as needed to help with carryover. This solution expires after six months.

60% Methanol, 20% Ultrapure Water, 20% Isopropanol
Combine 600 mL of methanol, 200 mL of ultrapure water, and 200 mL of isopropanol in an HPLC solvent bottle. This solution expires after six months.

0.1% Formic acid in 50% Methanol, 50% Ultrapure Water
Add 1 mL of concentrated formic acid to a 1:1 mixture of methanol and ultrapure water in an HPLC solvent bottle. This solution expires after six months.

95% LC Eluent "A" and 5% LC Eluent "B"
Combine 950 mL of LC Eluent "A" with 50 mL of LC Eluent "B" in an HPLC solvent bottle. This solution expires after one month.

9.16 LC-MS SMRM DRUG SCREEN IN URINE

Scope

This method is designed to screen and semi-quant members of a targeted list of analytes by tandem liquid chromatography-mass spectrometry scheduled multiple reaction monitoring (LC-MS sMRM) analysis.

This method is validated for use with urine specimens only. Calibrators, controls, and extracted blanks should be made in the appropriate matrix. A 50 µL aliquot may be used for analysis with a 1:1 dilution of blank urine. Alternatively an undiluted 100 µL aliquot of sample urine may be used if appropriate (DFSA case type).

This method is designed to detect the presence and perform semi-quantitation of targeted analytes. The analytes are separated from their matrix by liquid-liquid extraction, separated from one another by HPLC, and detected by tandem mass spectrometry, monitoring two to three transitions for the analytes and one transition for isotopically-labeled internal standards.

Chemicals and Reagents

- LC-MS grade formic acid (Fisher part A117-5D, or equivalent)
- Ultrapure water (17 MΩ-cm or greater)
- Ammonium formate (Fisher part A115-5D, or equivalent)
- LC-MS grade methanol (Fisher part A456-4, or equivalent)
- 96-well plate Nunc (Fisher part 12-565-6D6, or equivalent)
- Solutions A, B, C, Kura enzyme (provided by the kit manufacturer)

Controls

- Standards Test Mix
    - ToxBox (Screen)
    - 6-MAM, 7-aminoclonazepam, Acetyl fentanyl, Alprazolam, Amitriptyline, Amphetamine, Benzoylecgonine, Buprenorphine, Carbamazepine, Carfentanyl, Carisoprodol, Citalopram, Clonazepam, Cocaethylene, Cocaine, Codeine, Cyclobenzaprine, Dextromethorphan, Diazepam, Dihydrocodeine, Diphenhydramine, Doxepin, Doxylamine, Estazolam, Etizolam, Fentanyl, Flubromazolam, Flunitrazepam, Gabapentin, Hydrocodone, Hydromorphone, Levamisole, Loperamide, Lorazepam, MDA, MDMA, Meprobamate, Methadone, Methamphetamine, Midazolam, Mitragynine, Morphine, Norbuprenorphine, Nordiazepam, Norfentanyl, Norfluoxetine, Norquetiapine, Nortriptyline, Orphenadrine, Oxycodone, Oxymorphone, Paroxetine, Phencyclidine, Phentermine, Promethazine, Quetiapine, Sertraline, Tapentadol, Temazepam, Tizanidine, Tramadol, Trazodone, Triazolam, Venlafaxine, Zolpidem, Zopiclone

- Internal Standard Test Mix
    - ToxBox (Screen)
        - 6-MAM-D6, 7-aminoclonazepam-D4, Acetyl fentanil-D5, Alprazolam-D5, Amitriptyline-D3, Amphetamine-D11, Benzoylcegonine-D8, Buprenorphine-D4, Carbamazepine-13C6, Carfentanyl-D5, Carisoprodol-D7, Citalopram-D6, Clonazepam-D4, Clonazepam-D4, Cocaethylene-D3, Cocaine-D3, Codeine-D6,

Cyclobenzaprine-D3, Dextromethorphan-D3, Diazepam-D5, Dihydrocodeine-D6, Diphenhydramine-D3, Doxepin-D3, Doxylamine-D5, Estazolam-D5, Estazolam-D5, Etizolam-D3, Fentanyl-D5, Flunitrazepam-D7, Gabapentin-D1D, Hydrocodone-D6, Hydromorphone-D6, MDA-D5, MDMA-D5, Meprobamate-D7, Methadone-D9, Methamphetamine-D11, Midazolam-D4, Mitragynine-D3, Morphine-D6, Norbuprenorphine-D3, Nordiazepam-D5, Norfentanyl-D5, Norquetiapine-D8, Nortriptyiline-D3, Oxycodone-D6, Oxymorphone-D3, Paroxetine-D6, Phencyclidine-D5, Phentermine-D5, Promethazine-D3, Quetiapine-D8, Sertraline-D3, Tapentadol-D3, Temazepam-D5, Tizanidine-D4, Tramadol-13CD3, Trazodone-D6, Triazolam-D4, Venlafaxine-6, Zolpidem-D6, Zopiclone-D4

Equipment

- Micropipettes and tips
- 96-well plate shaker
- SPE dry 96-well plate evaporation workstation
- Heat sealer

Instrumentation

- Agilent 6460 C Series LC-MS Triple Quadrupole, AJS source
- Kinetex™ 2.6 µm Phenyl-Hexyl 100 Å, LC column 50x4.6 mm (Phenomenex part 00B-4495-E0 or equivalent)
- SecurityGuard™ ULTRA cartridges for Phenyl UHPLC (Phenomenex part AJ0-8774 or equivalent)

Instrument Conditions

- Injection volume: 5 µL
- Column oven: 25 °C

Time (min.)	Flow rate (µL/min.)	A%	B%
0.00	0.500	85.0	15.0
2.50	0.500	50.0	50.0
3.00	0.500	35.0	65.0
4.00	0.500	35.0	65.0
6.00	0.500	5.0	95.0
7.00	0.500	5.0	95.0
8.50	0.500	85.0	15.0
10.5	0.500	85.0	15.0

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Product Ion (m/z)	Product Ion (m/z)
6-MAM	328.2	165.1	211.1	
6-MAM-D6	334.2	165		
7-Aminoclonazepam	286.1	121.1	222.1	
7-Aminoclonazepam-D4	290.1	121.1		
Acetyl fentanyl	323.5	105.1	188.1	
Acetyl fentanyl-D5	328.5	104.9		
Alprazolam	309.1	281.1	205	
Alprazolam-D5	314.1	286.1		

Amitriptyline	278.2	91.1	105.1	
Amitriptyline-D3	281.2	91.1		
Amphetamine	136.1	91.1	119.1	
Amphetamine-D11	147.2	98.1		
Benzoylecgonine	290.1	168.1	77.1	
Benzoylecgonine-D8	298.1	171		
Buprenorphine	468.7	55.2	396	
Buprenorphine-D4	472.7	59.2		
Carbamazepine	237.1	194.1	193.1	
Carbamazepine-13C6	243	200.1		
Carfentanyl	395.5	113.1	105.1	
Carfentanyl-D5	400.5	113.1		
Carisoprodol	261.2	176.1	55	
Carisoprodol-D7	268.2	183.1		
Citalopram	325.2	109.1	262	
Citalopram-D6	331.2	109		
Clonazepam	316.1	270	214	
Clonazepam-D4	320	274		
Cocaethylene	318.2	82.2	77.1	
Cocaethylene-D3	321.2	199.2		
Cocaine	304.2	182.1	77.1	
Cocaine-D3	307.2	185.1		
Codeine	300.2	115.1	152.1	
Codeine-D6	306	152.1		
Cyclobenzaprine	276.2	215	216.1	
Cyclobenzaprine-D3	279.2	215.1		
Dextromethorphan	272.2	171.1	215.1	
Dextromethorphan-D3	275.2	171.1		
Diazepam	285.1	193	154	
Diazepam-D5	290.1	198.1		
Dihydrocodeine	302.2	199	128	
Dihydrocodeine-D6	308.2	202.1		
Diphenhydramine	256.2	167.1	165.1	
Diphenhydramine-D3	259.2	167.1		
Doxepin	280.4	107.1	77.2	
Doxepin-D3	283	107		
Doxylamine	271.4	167	182.1	
Doxylamine-D5	276.4	187.1		
Estazolam	295.1	267	205.1	
Estazolam-D3	346.1	317		
Etizolam	343.1	314	138.1	
Etizolam-D3	346.1	317		
Fentanyl	337.2	188.1	105.1	
Fentanyl-D5	342.3	105.1		
Flubromazolam	371	343	292.1	223.1
Flunitrazepam	314.3	268.1	239	
Flunitrazepam-D7	321.3	275.1		

Gabapentin	172.1	154.1	55.2	
Gabapentin-D10	182	147.2		
Hydrocodone	300.2	199.1	128.1	
Hydrocodone-D6	306.2	202.1		
Hydromorphone	286.2	185.1	157	
Hydromorphone-D6	292.2	185		
Levamisole	205.3	91.1	178	
Loperamide	478	267.2	266.7	
Lorazepam	321	275	229	
Lorazepam-D4	325.1	307		
MDA	180.2	163	135	
MDA-D5	185.2	168		
MDMA	194.2	163	105.1	
MDMA-D5	199.2	165		
Meprobamate	219.3	158.1	55.3	
Meprobamate-D7	226.3	165.1		
Methadone	310.2	265.1	105.1	
Methadone-D9	319.3	268		
Methamphetamine	150.1	119.1	91.1	
Methamphetamine-D11	161.2	97.1		
Midazolam	326.1	291.1	249.1	222.1
Midazolam-D4	330.1	295.1		
Mitragynine	399.5	174	159	
Mitragynine-D3	402.5	177.1		
Morphine	286.2	165	152.1	115.1
Morphine-D6	292	152		
Norbuprenorphine	414.3	83.2	55.2	
Norbuprenorphine-D3	417	55.2		
Nordiazepam	271.1	165	140	
Nordiazepam-D5	276.1	140		
Norfentanyl	233.3	84.2	55.3	
Norfentanyl-D5	238.3	84.2		
Norquetiapine	296.4	253	210	
Norquetiapine-D8	304.4	210		
Nortriptyline	264.2	105.1	91.1	
Nortriptyline-D3	267	91.1		
Orphenadrine	270.4	181	165	
Oxycodone	316.2	298.1	241.1	
Oxycodone-D6	322.2	304.1		
Oxymorphone	302.1	284.1	227.1	
Oxymorphone-D3	305.2	287.1		
Paroxetine	330.4	192.1	70.2	
Paroxetine-D6	336.4	76		
Phencyclidine	244.4	91.1	86.2	
Phencyclidine-D5	249.4	86.2		
Phentermine	150.2	91.1	65.2	
Phentermine-D5	155.2	96.1		

Promethazine	285.1	86.2	71.2	
Promethazine-D3	288.2	89.2		
Quetiapine	384.2	253	221.1	
Quetiapine-D8	392.2	226		
Sertraline	306.1	275	159	
Sertraline-D3	309.1	275		
Tapentadol	222.3	107.1	77.2	
Tapentadol-D3	225.3	107.1		
Temazepam	301.1	283	255.1	
Temazepam-D5	306.1	260.1		
Tizanidine	254.7	69	44.3	
Tizanidine-D4	258.1	48.2		
Tramadol	264.2	58.2	42.2	
Tramadol-13C-D3	268.2	58.2		
Trazodone	372.2	176.1	148.1	
Trazodone-D6	378.2	182.1		
Triazolam	343.1	315	308.1	
Triazolam-D4	347.1	319		
Venlafaxine	278.2	260.2	58.2	
Venlafaxine-D6	284.2	64.2		
Zolpidem	308.2	235.1	65.2	
Zolpidem-D6	314.2	235.1		
Zopiclone	389.1	245	112	
Zopiclone-D4	393.1	245		

Source Parameters:

Parameter	Value (+)
Gas Temperature (°C)	350
Gas Flow (l/min)	10
Nebulizer (psi)	45
Sheath Gas Temp (°C)	380
Sheath Gas Flow (l/min)	11
Capillary (V)	3500
Nozzle Voltage/Charging (V)	0

Procedure:

1. Allow 96 well standard plate (custom prepared analytes plate), urine, and samples to reach room temperature.
2. Pipette 100 µL blank urine into wells containing standards and QCs.
3. Pipette 50 µL blank urine into wells containing only internal standards (sample wells) for sample dilution.[26]

[26] If using undiluted sample, pipette 100 µL of sample into wells containing only internal standard. Then proceed to step 5.

4. Pipette 50 µL of sample urine into wells containing only internal standards and blank urine (sample wells).
5. Add 100 µL of Kura Enzyme to all wells of plate.
6. Add 200 µL of DI water to each well.
7. Place on the shaker at 900 rpm for 30 minutes at room temperature.
8. Add 100 µL of Solution A to each well.
9. Place on the shaker at 900 rpm for 5 minutes at room temperature.
10. Add 650 µL Solution B. Mix via aspirate and dispense approximately 10 times.
11. Allow plate to sit for 15 minutes.
12. Remove the bottom layer by aspirating 500 µL with tips against the well bottom. Wait for 2-3 seconds before lifting pipette. Discard bottom layer to waste.
13. Place plate on SPE evaporator at 35°C and evaporate to dryness. As the plate nears complete evaporation, the temperature may be increased to no more than 60 °C.
14. Reconstitute with 500 µL of Solution C and heat seal the plate with foil.
15. Store the plate at 4°C until ready to run the sequence.

Quality Assurance, Interpretation, Precautions, and Notes
This analytical technique does not produce a diagnostic mass spectrum (like that typical of El GCMS), so a different type of assessment is necessary to determine whether a targeted analyte is present. In order to report a "positive" result, the following criteria must be met:

Calibrators:
Up to four calibrators will be analyzed to set the linear range of the method. These calibrators will be prepared in a ToxBox. The concentrations of these calibrators will vary by analyte, with the expected working range listed in the validation document for each analyte. Calibrators outside of the validated working range may not be included in the assay.

The calibration curve should meet the requirements outlined in§ *7.7.1, Calibration Curves* excluding r-values of curves. The correlation coefficient of each analyte must be ≥0.85.

Controls:
The concentration of the positive controls will vary by analyte, depending upon the expected working range of the assay for that analyte. Typically, both positive control concentrations will be analyzed to evaluate the working range. Both positive controls should fall within ±50% of their target value and be within the working range of the calibration curve.

A negative control will be extracted (containing only internal standard) to demonstrate the absence of any contaminant which would result in a false positive response. The targeted analyte must not be detected (i.e., reportable) in the negative control.

Specimens:
One aliquot of each case specimen is typically run. A 1:1 dilution of each case specimen with blank urine (i.e. human urine from Golden West or the equivalent) may be used-no other diluent may be used to dilute the specimen.

Signal-to-Noise Ratio (S:N):
The signal-to-noise ratio for each transition must be greater than 8:1. If no background noise exists for a given transition, then this requirement is considered to be met. Interfering peaks within the collection window may result in a lower signal-to-noise ratio for the analyte. When there is an interfering peak, analytes with a S:N ≤ 8 may be reported if all other criteria are met (i.e., concentration at or above the decision point, proper integration of peaks, and correct relative retention time). If the S:N is ≤ 5 due to an interfering peak, reporting that analyte requires the written approval of the Chief Forensic Toxicologist.

Retention Time
The retention time must be within ±2% of the expected relative retention time, which is established by a certified reference material. It is defined as the quotient of the retention time of the analyte and of the internal standard. The expected relative retention time is set in the instrument method, but may be evaluated on a batch-by-batch basis. An exception will be made for analytes that do not have an isotopically-labeled internal standard or specific analytes that are more suitable to a surrogate internal standard (e.g., Etomidate with Alprazolam-D5). Relative retention time differences greater than ±2% may occur due to large analyte concentrations present in urine. In these cases, values greater than ±2% but less than ±4% may be acceptable with the written approval of the Chief Forensic Toxicologist. Exceptions will be made for gabapentin and methamphetamine due to routine large analyte concentrations present in urine where RRT differences for these two analytes do not require approval by the Chief Forensic Toxicologist.

Other notes and requirements:
The qualitative presence of each analyte is determined by the evaluation of the following parameters:

- Concentration of the analyte at or above the decision point
- Integration of transition(s)
- Relative retention time (RRT)
- Signal to noise (S:N)

This semi-quantitative analysis provides an estimated analyte concentration which will be used to interpret the presence or absence (not detected) of the analyte.

An analyte whose concentration is at or above the decision point will be reported as present. If a sample has a drug concentration below the analytes' decision point, it will not be reported. This was administratively set for all analytes in the method validation.

Chromatographic peaks are automatically integrated during data analysis. It is the responsibility of the analyst to ensure that all appropriate chromatographic peaks are integrated. Analysts may sometimes need to adjust automatic integrations to properly represent the analytical data. Examples of these actions include: integrating the correct analyte among isobars, accounting for the qualifier ion for an analyte at a low concentration, and correcting an incomplete peak integration. A manual integration of apparent noise is not necessary to meet acceptability criteria to report an analyte in a sample or the associated case blank.

Sample plates should be run ideally within the first 7 days after extraction if stored refrigerated at 4°C. Sample plates may be stored at 4°C for up to 19 days after extraction if necessary. Sample

plates analyzed beyond the recommended 7 days require the technical batch review to be completed by the section Technical Leader, Quality Manger, or Chief Forensic Toxicologist.

Solvent blanks or extracted blanks may be utilized to evaluate carryover. Suspected carryover peaks for reportable analytes must be evaluated by the following:

Solvent blank:

a) Less than 5% of the area of the peak for which it is blank, or
b) Less than 50% of the area of the lowest calibrator

Extracted blank:

a) The amount of analyte present in the blank cannot exceed the decision point
b) Elevated results (i.e., area of an analyte in the blank ≥50% of the area of the decision point) in blank injections following samples containing high amounts of analyte are evaluated on a case-by-base basis, and may require re-extraction or reanalysis before reporting.

The performance of calibrators and controls is evaluated by a second analyst, and a record of this evaluation is recorded on a batch worksheet and maintained in the case record.

The SPE dry evaporation workstation is the only appropriate evaporation manifold that may be used for this method. The TurboVap evaporation workstation will not be utilized with this method due to carryover.

Preparation of Materials

LC Eluent "A"
Add 1 mL of concentrated formic acid to 0.95 grams ammonium formate, and bring to volume with 1 L of ultrapure water in a 1 L class A volumetric flask. This solution expires after one month.

LC Eluent "B"
1 L of HPLC grade methanol. This solvent does not expire.

Needle Wash
100 mL ultrapure water, 200 mL HPLC grade acetonitrile, 200 mL HPLC methanol, 500 mL HPLC isopropanol. This solution expires after 6 months.

95% LC Eluent "A" and 5% LC Eluent "B"
Combine 950 mL of LC Eluent "A" with 50 mL of LC Eluent "B" in an HPLC solvent bottle. This solution expires after one month.

100% Methanol
1 L of HPLC grade methanol is added to an HPLC solvent bottle. This solvent is used to help with carryover and does not expire.