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URINE DRUG ANALYSIS

Purpose

Drugs that impair performance can be detected in blood, urine or other biological specimens. Samples of the biological specimen are screened for the presence of classes of compounds. A sample is extracted and purified for compound confirmation on the gas chromatograph mass spectrometer (GC/MS). Comparison of mass spectral characteristics in conjunction with the chromatographic retention time provides qualitative identification.

Safety

Observe Universal Bloodborne Pathogen Precautions. Personal protective equipment during sample preparation should include: eye protection, lab coat, non-porous gloves (latex, nitrile, neoprene, etc.), and a biosafety cabinet.

A hepatitis B inoculation is recommended for anyone handling biological fluids.

Use appropriate safety equipment when preparing reagents and handling volatile or caustic chemicals. Refer to MSDS for additional safety information for specific chemicals.

Equipment and Supplies

- Immunoassay test panel
- Toxi-Lab supplies and chemicals
- Appropriate Internal Standard
- **Urine controls**
  - Negative urine controls – commercially prepared
  - Positive urine controls – commercially prepared
- Gas chromatograph mass spectrometer (GC/MS)
- Vortex
- Centrifuge
- Heat block
- Evaporator
- Air displacement pipette and disposable tips
Disposable pipettes
Auto sampler vials with micro volume inserts and crimp caps
Glassware (culture tubes, centrifuge tubes, micro reaction vials, etc.)

Analysis of Specimens Containing Sufficient Volume (more than 20 ml)

Allow all specimens to equilibrate to room temperature before analysis. Ensure that sufficient sample volume remains to allow for independent analysis.

Screening

• Immunoassay Screening

Perform an immunoassay screen for the presence of cannabinoids, benzodiazepines, and other drugs as needed or provided on the test panel.

1. If the cannabinoid, cocaine, opiates, or benzodiazepine assay indicates a positive response, follow the specific hydrolysis, extraction, and derivatization procedures for confirmation. Other positive responses are confirmed by regular procedures. If multiple positive responses are indicated (i.e. benzo+cannabinoids), one procedure may be performed if all identified analytes can be confirmed in that procedure.

2. A negative response indicates additional procedures need not be performed for that analyte or class of analyte.

3. Negative and positive controls will be run with all batched urine casework specimens.

• Toxi-Lab Screening

Follow the Toxi-Lab procedures for extracting, inoculating, development, and detection of possible acidic, neutral and/or basic drugs in the urine specimen.

1. Toxi-Lab A Extraction – All samples deemed appropriate by the analyst (e.g. if performing Toxi-Lab will give a preliminary or secondary confirmation)

2. Toxi-Lab B Extraction – If immunoassay is positive for barbiturates

3. Toxi-Lab Benzoylecgonine (BE) Extraction – If immunoassay is positive for cocaine metabolites

4. Negative and positive controls will be run with all batched urine casework specimens.
TSD Detector

The TSD detector on the Varian 4000 may be utilized as a screening mechanism on urine specimens. Basic urine extraction schemes will apply with negative and positive controls run with all specimens analyzed.

Confirmation

GC/MS

1. Extract a second sample using the specific Toxi-Lab extraction tube for the detected analyte (e.g. for confirmation of sympathomimetic amines, use Toxitube A) or Solid Phase Extraction (SPE) cartridge utilizing separate acid/base fractions. Extraction protocol will follow UCT-SPE method.

2. The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 12)

3. If confirming volatile compounds (e.g., amphetamines, methamphetamine, pseudoephedrine, etc.) before complete drydown of extraction solvent, add 100µl of 10% HCl/MeOH, or stop short of complete drydown leaving approximately 100-200 µl of solvent.

4. Reconstitute the residue with an appropriate organic solvent and place into a crimped auto sampler vial for GC/MS analysis. Volume reducers may be used as needed.

5. Negative and positive controls will be run with all batched specimens analyzed to include general extraction and special extractions (i.e. benzodiazepines, opiates, benzoylecgonine, etc.)

6. Document on the data entry: method name; description of sample fractions analyzed; analysis procedure; derivatizing reagent used.

GC Retention Time

1. Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory.

2. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed. Retention time is determined at the peak maximum height. If the peak width exceeds 0.6 minute, then the sample is diluted and re-analyzed before the retention time may be used.
Benzodiazepine/Opiates Derivatization Procedure

When the benzodiazepine immunoassay indicates positive, sample extraction is needed for confirmation:

1. If needed, adjust the pH of the sample with 0.1 M acetic acid to ensure that it is acidic.
2. Hydrolyze 5 ml urine with β-glucuronidase for 2-3 hours at 55° C.
3. The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 12).
4. Extract with the appropriate method (Toxi-Tube A, Toxi-Tube B, or SPE).
5. Evaporate the extract into a micro reaction vial and reconstitute with ethyl acetate and MSTFA.
6. Heat at 60° C for a minimum of ½ hour.
7. Place the sample into a volume reducing vial, crimp, then inject on the GC/MS.

Carboxy THC Derivatization Procedure

When the cannabinoid immunoassay indicates positive, sample extraction is needed for confirmation:

1. Hydrolyze 3-5 ml urine with 200 µl of 10 N NaOH at room temperature for 15 minutes.
2. The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 12).
3. Adjust the pH to ~4-5 using glacial acetic acid.
4. Extract using Toxi-Tube B.
5. Evaporate the extract into a micro reaction vial and reconstitute with ethyl acetate and MSTFA.
6. Heat at 60° C for a minimum of ½ hour.
7. Place the sample into a volume reducing vial, crimp, then inject on the GC/MS.
Toxicology

- **Toxi-Lab Benzoylecgonine Extraction and Derivatization**

  When the immunoassay panel screen indicates the presence of benzoylecgonine (cocaine metabolite), sample extraction is needed for confirmation. Perform a Toxi-Lab Benzoylecgonine Derivatization Procedure. With the same Toxi-Lab A extraction tube used to extract a second sample for Toxi-Lab screening, perform the benzoylecgonine extraction of the remaining aqueous layer.

  1. Using the same Toxi-Lab A extraction tube used for Toxi-Lab screening, add methylene chloride to fill to level.
  2. The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 12).
  3. Mix by inversion and extract the solvent.
  4. Evaporate the extract into a micro reaction vial and reconstitute with ethyl acetate and MSTFA.
  5. Heat to 60° C for a minimum of ½ hour.
  6. Place the sample into a volume reducing vial, crimp, then inject on the GC/MS.

- **Gamma Hydroxybutyrate (GHB) Screening (by special request only)**

  Perform analysis on sexual assault cases requested by the case officer and special cases as requested by DRE officers.

  1. GHB Liquid-Liquid Extraction Procedure
  2. Gamma Hydroxybutyrate Derivatization Procedure

- **Toxi-Lab Special and Validation Procedures**

  Consult the Toxi-Lab Drug Compendium for specific procedures. Call the Toxi-Lab hotline (800-322-3443) for assistance.

**Specimens Containing Insufficient Volume (less than 20 ml)**

Tests to be performed will change due to conditions and requirements of each case. Ensure that sufficient sample volume remains to allow for independent analysis.
Determine tests to be performed

Review the submission form for possible drug indications. Some tests may be eliminated due to an insufficient volume of sample available. Selection of tests will be dependent on the drugs indicated by the officer.

Screening

- Immunoassay screening
  Test for the drugs provided on the immunoassay panel if sufficient sample is available. Negative and positive controls will be run with all batched specimens analyzed. The assay of choice for insufficient volume samples will be the Triage or BioRad Tox/See Immunoassay. It may be necessary to be selective in the choice of the test(s) based on indications from the submission form (or conversation with the officer) and the volume of specimen present.

- Reduced Volume Toxi-Lab Screening
  1. Add ~ 3 ml of urine to a Toxi-Tube A extraction tube.
  2. Fill the tube to the 5 ml line with water, if needed.
  3. Extract and develop according to Toxi-Lab procedures for the presence of basic drugs. It may be necessary to hydrolyze the specimen with β-glucuronidase before extracting.
  4. Perform Toxi-Lab extraction for benzoylecgonine if indicated by a positive for cocaine metabolite on the immunoassay panel.
  5. Perform a Toxilab B extraction if indicated by a barbiturate positive on an immunoassay panel.
  6. If a different class of drug is indicated (i.e. acidic, cannabinoids, GHB, etc.), it may be necessary to perform the specific extraction rather than a general extraction.
  7. Negative and positive controls will be run with all batched specimens analyzed.

Confirmation

- GC/MS

  If insufficient sample is available for reanalysis, the analyte(s) cannot be confirmed nor reported.
If sufficient sample is available (more than 10 ml), extract a second sample, using the specific Toxi-Lab extraction tube for the detected analyte (e.g. for confirmation of carisoprodol, use Toxi-Lab A) or solid phase extraction (SPE) as per UCT extraction protocol. Negative and positive controls will be run with all batched specimens analyzed to include general extraction and special extractions (i.e. benzodiazepines, opiates, THC, etc.). The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 12).

1. If confirming for volatile compounds, e.g. amphetamines, methamphetamine, pseudoephedrine, etc., add 100 µl of 10% HCl/MeOH before evaporation, or stop short of complete drydown by leaving 100-200 µl of solvent.

2. Reconstitute the residue with an appropriate organic solvent and place into a crimped auto-sampler vial for GC/MS analysis.

3. Document on the data entry: method name; description of sample fractions analyzed; analysis procedure; and derivatizing reagent used.

4. Perform benzodiazepine and/or cannabinoid confirmation, if needed. It may be necessary to extract with reduced volume.

- **GC Retention Time**

Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed.

- **Benzodiazepine Derivatization Procedure**

When the benzodiazepine immunoassay indicates positive, sample extraction is needed for confirmation. Perform extraction if additional sample is available.

- **Carboxy THC Derivatization Procedure**

When the cannabinoid immunoassay panel indicates positive, sample extraction is needed for confirmation. Perform extraction if additional sample is available. Use the base hydrolysis and Toxi-Tube B extraction given previously.

- **Toxi-Lab Benzoylecgonine Extraction and Derivatization**

When the immunoassay panel screen indicates positive cocaine, sample extraction is needed for confirmation.
If solid phase extraction is required for benzodiazepine derivatization, benzoylecgonine may be detected through this method. If benzodiazepine is not detected, confirm by the previously listed procedure.

- **Gamma Hydroxybutyrate (GHB) Screening (by special request only)**
  Perform if additional sample is available.
  1. GHB Liquid-Liquid Extraction Procedure
  2. Gamma Hydroxybutyrate Derivatization Procedure

- **Toxi-Lab Special and Validation Procedures**
  Perform if additional sample is available. Consult the Toxi-Lab Drug Compendium specific procedures. Call the Toxi-Lab hotline (800-322-3443) for assistance.

**Standards and Controls**

Before being placed into service, all urine controls will be verified using established laboratory procedures and expiration dates verified. For MSTFA derivatizing reagent – expiration date will be three years from the date of purchase. For in-house controls – three years from the date of preparation.

Obtain pharmaceutical standards or pure commercially prepared standards for comparison of instrumental spectra or chromatographic characteristics, if the compound of interest is not available in reference libraries.

Check the blank result analyzed before each case sample on the GC/MS. If a blank contains presence of analytes, the case sample analysis is suspect and must be reanalyzed.

**Data Analysis**

Qualitative identification of an analyte is based on detection by two dissimilar methods of analysis. Retention times from the case sample must match that of the standard to within ± 0.125 minutes. If the analyte cannot be confirmed by two different methodologies due to a lack of available methods, confirm by GC/MS with extraction of a second sample by the same procedure as used for the screen or use a specific extraction procedure. Confirmation must be performed on a sample aliquot taken from the specimen that was not used to originally screen for analytes.

The mass spectrum of the analyte must compare to that for the standard obtained through analysis on laboratory instruments or spectra from literature or reference material. A spectrum is considered acceptable if all major ions are readily identified.
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without the presence of any interfering ions from another analyte (i.e. co-elution). If other interfering ions are present, the analyst must consult with another analyst in the section to determine if the analyte in question can be reported. This consultation must be documented on the worksheet. If the spectrum is not acceptable, it will not be used for confirmation. Place all spectra in the case folder.

The limit of detection for an analyte shall be defined as the concentration of the analyte required to give a signal equal to the background plus three times the standard deviation of the background.

If the mass spectrum reveals analytes that were not identified by immunoassay or toxilab screening, verify that the analyte is not from carryover from another specimen. Carryover may occur due to the use of automated GC injection systems or due to the extreme range of drug concentration detected in toxicological specimens. Regardless of cause, extreme caution is warranted when carryover is detected and requires sectional notification and review of analytical results. Appropriate action to address carryover may include reinjection with added solvent blanks between specimens or reanalysis of some or all specimens. If carryover can be ruled out, focus should be redirected to the screening mechanisms. If negative and positive controls used in batch analysis have proven to have acceptable responses, the immunoassay or toxilab cutoff levels may be higher than the quantity of analyte in the specimen. In this case, use of a specific extraction procedure on another aliquot of the specimen should be used for confirmation of the analyte.

Reporting

All printouts, spectra, and reports of analysis must be included in the case folder. Toxi-Lab observations must be drawn on the Toxi-Lab worksheets. A TLC may be photocopied or the observation will be noted as matching the corresponding standard. Case identifiers and analyst’s handwritten initials must be present on all sheets.

If sufficient data supports identification of an analyte, it will be qualitatively listed as present. Any metabolites detected may be reported as a metabolite of a specific parent drug (e.g. codeine metabolite), or simply identified by name (e.g. α-hydroxyalprazolam, norcodeine).

An analyst who is proficient in toxicology will administratively and technically review the case folder. The technical review will be documented in the LIMS.
Securing Evidence

All evidence shall be sealed, dated and initialed prior to being released to the evidence section for filing and storage.

References

- SOFT/AAFS Forensic Toxicology Laboratory Guidelines (available on-line)
- Toxi-Lab Training Manual
- United Chemical Technologies Applications Manual (UCT) (available on-line, on the Toxicology computer, and on disk)
Trimethysilyl Derivatization Procedure

Certain drugs are not readily analyzed by GC/MS. They may be too polar, thereby giving broad, ill-defined peaks. Most of these drugs may be made more stable or less polar by derivatization. The drugs that readily derivatize with silation reagent will have an acid, alcohol, or secondary amine functional group.

Sample Hydrolysis and Extraction:

- Extract from matrix by the approved method for the specific drug. See specific extraction method for the drug of interest.
- Place extract into a micro reaction vial.
- Evaporate organic solvent to dryness. Do not use any type of aqueous extract, since water reacts readily with the derivatizing reagent.

Derivatization:

- Reconstitute the extract in ethyl acetate (~ 50 μL for urine; 50 μL for blood)
- Add derivatizing reagent (~50-150 μL for urine; ~50 μL for blood) to the residue. Either MSTFA or BSTFA may be used.
- Place the micro reaction vial in an oven or dry block heater at 60 °C for 15 minutes. If clonazepam is suspected, heat for one hour.

Analysis:

Inject 1 μL of the reaction mixture into the GC/MS, using the BENZOTMS OR BLDDERIV-MS method.
Internal Standard Procedure

Appropriate Internal Standards should be added to toxicology samples to verify completeness and precision of the various extraction methods utilized in the section. The two main extraction techniques utilized are solid phase and liquid/liquid. An aliquot of prepared ISTD should be added to all controls and case samples to verify extraction completeness. Additionally, as other components are incorporated into sample extraction, such as quantitation procedures, the ISTD method may be useful in helping verify extraction proficiency and instrumental quantitative precision.

All internal standards utilized should be verified by existing QA/QC measures before being placed into service. If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and add quantity sufficient to bring up to volume with methanol, acetonitrile, or appropriate solvent (qs). Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for three (3) years.

From the stock solutions, various working solutions may be made:

<table>
<thead>
<tr>
<th>Working solution concentration</th>
<th>amt stock used</th>
<th>volumetric flask dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>100μg/mL</td>
<td>1mL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>50μg/mL</td>
<td>500μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>20μg/mL</td>
<td>200μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>10μg/mL</td>
<td>100μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>5μg/mL</td>
<td>50μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>2μg/mL</td>
<td>20μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
</tbody>
</table>

The equation to figure working solution concentration when starting with a stock solution of 1mg/mL is as follows: (amount of stock solution used in mL) / (final volume in mL) * 1000. Therefore, if 250μL of stock solution is added to 10mL volumetric flask (qs) – the resulting concentration of the solution is 0.250/10*1000 = 25μg/mL.

If different stock concentrations other than 1mg/mL are used, adjust the amount of stock used accordingly. For instance, if a concentration of stock is purchased at a level of 100μg/mL – the resulting measures take effect.
Working solution concentration  | amt stock used | volumetric flask dilution  
--- | --- | ---  
50μg/mL  | 2.5mL | 5mL qs w/ MeOH  
20μg/mL  | 1mL | 5mL qs w/ MeOH  
10μg/mL  | 500μL | 5mL qs w/ MeOH  
5μg/mL  | 250μL | 5mL qs w/ MeOH  

Internal standard concentrations for casework samples is therefore dependent on which working solutions the analyst will be using and the amount of specimen used from the casework sample. In other words, the amount of internal standard depends on the amount of ISTD added in μL and the concentration of the ISTD added (usually μg/mL). For instance, if 30μL of a 20μg/mL ISTD solution added to 1mL sample of a negative blood control, then a total of 0.6μg of ISTD is in the negative control (0.03 * 20 = 0.6). Since the final volume is theoretically 1mL, then this control has an ISTD concentration of 0.6μg/mL. Another example would be if 100μL of a 20μg/mL ISTD solution is added to 1mL of a negative blood control, then the total ISTD present in the sample is 2μg (0.1 * 20 = 2μg). Since the final volume 1mL, then this control has an ISTD concentration of 2μg/mL.

Therefore, starting with ISTD working solution of 5μg/mL or 10μg/mL, the following concentration levels may be attained. All of the following solutions will be in 1mL negative blood control. Remember these will be theoretical calculations only. The amount of ISTD solution should be added to an amber screw top vial and evaporated under nitrogen before the control blood is added.

| Concentration of ISTD in sample | mL of ISTD control to be used |
| --- | --- | --- |
|  | (5μg/mL) | (10μg/mL) |
| 1.0μg/mL also 1000ng/mL | 200μL | 100μL |
| 0.5μg/mL also 500ng/mL | 100μL | 50μL |
| 0.25μg/mL also 250ng/mL | 50μL | 25μL |
| 0.10μg/mL also 100ng/mL | 20μL | 10μL |
| 0.05μg/mL also 50ng/mL | 10μL | 5μL |
| 0.025μg/mL also 25ng/mL | 5μL | 2.5μL |
| 0.01μg/mL also 10ng/mL | 2μL | 1μL |
| 0.005μg/mL also 5ng/mL | 1μL | 0.5μL |

PPDLAB Toxicology Casework Samples:

Present volumes of samples used for toxicology exam include using 2mL of urine or 1 to 1.5mL of blood. Therefore, 40μL of a 5μg/mL ISTD solution added to 2.0mL of urine would yield a concentration of 0.10μg/mL or 100ng/mL and 30μL of a 5μg/mL ISTD solution added to 1.5mL of blood would yield a concentration of 0.10μg/mL or 100ng/mL.
100μg/mL. If more specialized extraction techniques are utilized such as urine cannabinoids or benzodiazepines, the following additions should be added: For urine cannabinoids using 5mL of urine, use 100μL of a 5μg/mL ISTD solution. For urine benzodiazepines using 5mL of urine, use 100μL of a 5μg/mL ISTD solution.

### INTERNAL STANDARDS CHART TO PREPARE A WORKING CONCENTRATION OF 5μg/mL

<table>
<thead>
<tr>
<th>Extraction - SPE or liq/liq</th>
<th>ISTD used (powder/base/conc)</th>
<th>Amount stock used for 5μg/mL working</th>
<th>Volume qs</th>
<th>Manufacturer</th>
<th>Product #</th>
<th>Date rec'd</th>
<th>Lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic</td>
<td>Thiopental - 1mg/mL</td>
<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>T1019-10MG</td>
<td>6/20/2010</td>
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<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>M3189-1G</td>
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<td>030M1223</td>
</tr>
<tr>
<td></td>
<td>SKF-38393 HCl - 1mg/mL</td>
<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>D047-500MG</td>
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<tr>
<td>Benzodiazepines</td>
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<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>P3654-25MG</td>
<td>6/20/2010</td>
<td>056F0684</td>
</tr>
<tr>
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<td>500μL</td>
<td>10mL</td>
<td>Cerilliant</td>
<td>H041</td>
<td>3/26/2010</td>
<td>FE083107-02</td>
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</tbody>
</table>
BLOOD DRUG ANALYSIS PROCEDURE

Purpose

Drugs can be detected by analyzing blood, urine or other biological specimens. A sample is extracted and purified for analyte confirmation on the gas chromatograph mass spectrometer (GC/MS) and with other techniques. Comparison of mass spectral characteristics of the sample to a standard provides qualitative identification, in conjunction with the chromatographic retention time.

Safety

Observe Universal Bloodborne Pathogen Precautions. Personal protective equipment during sample preparation should include: eye protection, lab coat, non-porous gloves (latex, nitrile, neoprene, etc.), and a bio-safety laminar flow hood.

A hepatitis B inoculation is recommended for anyone handling biological fluids.

Use appropriate safety equipment when preparing reagents and handling volatile or caustic chemicals. Refer to MSDS for additional safety information for specific chemicals.

Equipment and Supplies

- Solid Phase Extraction Columns
- Gas chromatograph mass spectrometer (GC/MS)
- Vortex
- Centrifuge
- Evaporator
- Air displacement pipette and disposable tips
- Disposable pipettes
- Auto sampler vials with micro volume inserts and crimp caps
- Glassware (culture tubes, centrifuge tubes, micro reaction vials, etc.)
- **Blood drug controls**
  - **Negative blood drug control** – commercially prepared such as ethanol whole blood control or volatile whole blood control or other available whole blood control
  - **Positive blood drug control** – commercially prepared such as ethanol whole blood control spiked with an in-house prepared drug mix documented with appropriate paperwork.
The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16).

Specimens Containing Sufficient Volume: > 3 ml available

Allow all specimens to equilibrate to room temperature before analysis. Ensure that sufficient sample volume remains to allow for independent analysis.

Blood alcohol concentration

If the blood alcohol concentration is > 0.08 grams/100 ml of blood, no further analysis is necessary. However, a drug screen will be performed if requested by the submitting officer in certain instances, i.e. sexual assaults, vice, burglary & theft, DWI manslaughter, etc. If the blood alcohol concentration is < 0.08 grams/100 ml of blood, analyze for the possible presence of drugs.

See Ethanol Quantitation Procedure.

General Drug Extraction – Preliminary Identification

Solid Phase Extraction of Blood Sample

Perform analysis using the United Chemical Technologies procedure for "Forensic Sample Drug Analysis for GC or GC/MS using: Xtract® column XRDAH206 (large particle size and high porosity frit) or XRDAH 505." Follow sample preparations for whole blood. Allow gravity or positive pressure to force the sample and reagents to flow through the SPE tube.

- Place the reconstituted residue into a crimped auto sampler vial for GC/MS analysis, using a general or toxicology method.
- Document on the data entry: method name, description of sample fractions analyzed, analysis procedure and derivatizing reagent.
- When the GC/MS is complete for Fractions A and B, Combine both Acid/Neutral and Basic Fractions to derivatize with MSTFA. See the Trimethyl Silyl Derivatization Procedure. Inject the derivatized sample into the GC/MS, using the BENZOTMS or BLDDERIV-MS method. Derivatizing the fractions will enable identification of benzodiazepines, opiates, etc. which may be undetectable when underivatized.
Liquid/liquid Extraction

- Use Toxi-Lab A/B extraction procedures following the manufacturer’s instructions. Negative and positive controls will be run with all batched blood samples analyzed. (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Addition Procedure (pg. 15)).

Interpretation of results:

Screening – GC retention time
Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed.

Secondary Screening – TSD Detector
The TSD detector on the Varian 4000 may be utilized as a secondary screening mechanism on blood samples. The extraction mechanism will follow the general drug extraction routine with proper negative and positive control runs. Retention times from the case sample must match that of the standard to within ± 0.150 minutes. A list containing retention times is maintained in the laboratory.

Preliminary Identification – Mass Spectrum
The mass spectrum conditionally identifies the compound.

Confirmation Extraction
1. Once conditional identification has been established, a second extraction from the sample exhibit shall be performed. This extraction should confirm the results from the conditional extraction and allow the reporting of the analyte. Ideally, the second sample should be from a different sample exhibit than the first sample; however, if this is not possible, a different aliquot from the initial sample may be used. The extraction procedure may be the same as initially used (general drug extraction) or a more specialized version (e.g. opiate, benzodiazepines) as highlighted in the United Chemical Technology Manual. This extraction should also encompass the use of positive and negative controls. (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16)).
If sufficient sample is unavailable for a confirmation extraction and the preliminary ID is from Mass Spectra analysis, the analyte may be reported as a preliminary result.

- Preliminary Mass Spectra analysis of the submitted sample did indicate the presence of drug A, however due to insufficient sample volume the confirmation of drug A could not be reported.

**Gamma Hydroxybutyrate (GHB) [by special request only]**

A solid phase method for GHB extraction (United Chemical Technologies)

- Pipette 200 µL of unknown blood sample into appropriately labeled 1.5 ml plastic centrifuge tube.
- Add 1 ml of acetone; vortex 15 seconds.
- Centrifuge; transfer acetone layer to culture tubes.
- Evaporate extracts
- Reconstitute the dried extracts with 200 µL of 0.1 Phosphate Buffer pH 6.0 buffer; vortex 15 seconds.
- Prepare Clean Screen® GHB (200 mg in a 10 ml tube)
  - Extraction columns as follows:
    1 x 3 ml of CH₃OH; aspirate.
    1 x 3 ml of DI H₂O; aspirate.
    1 x 3 ml of 0.1 M Phosphate Buffer (pH 6.0); aspirate.
  - **Note:** Aspirate using vacuum. Avoid drying sorbent completely.
- Apply sample with disposable pipette
- Elute GHB – Add 1 ml of CH3OH/NH4OH (99:1) to original sample test tube; vortex. Decant onto column and collect extract. Aspirate ~1 inch Hg.
- Concentrate – Remove test tube containing eluate. Evaporate to dryness.
- Derivatize – Add 100 µL of ethyl acetate and 100 µL of MSTFA with 1% TCMS. Mix/Vortex. No heating required.
- Inject on the GC/MS using the appropriate method.
- Can also extract a 2 ml sample of the whole blood two times with chloroform. Combine the chloroform extracts and evaporate to dryness with nitrogen. Reconstitute with 10 µL ethyl acetate and 10 µL MSTFA or BSTFA + 1% TMCS. See GHB Derivatization Procedure.
- Positive and negative controls will be utilized with all GHB batch sample runs. The negative control will be commercially prepared while the positive control will be a commercially prepared whole blood base spiked with an in-house standard of GHB.
Interpretation of results:

Screening – GC retention time
Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed.

Preliminary Identification – Mass Spectrum
The mass spectrum conditionally identifies the compound.

Confirmation Extraction
1. Once conditional identification has been established, a second extraction from the sample exhibit shall be performed. This extraction should confirm the results from the conditional extraction and allow the reporting of the analyte. Ideally, the second sample should be from a different sample exhibit than the first sample; however, if this is not possible, a different aliquot from the initial sample may be used. The extraction procedure may be the same as initially used (general drug extraction) or a more specialized version (e.g. opiate, benzodiazepines) as highlighted in the United Chemical Technology Manual. This extraction should also encompass the use of positive and negative controls. (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16)).

If sufficient sample is unavailable for a confirmation extraction and the preliminary ID is from Mass Spectra analysis, the analyte may be reported as a preliminary result.

- Preliminary Mass Spectra analysis of the submitted sample did indicate the presence of GHB, however due to insufficient sample volume the confirmation of GHB could not be reported.

THC and Carboxy THC in Whole Blood for GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column [to be performed on all samples with sufficient volume]
Procedure from United Chemical Technologies, Inc.:

- To 1 ml of whole blood add 2 ml of acetonitrile, vortex for 30 seconds and allow to stand for 3 minutes. Centrifuge for 10 minutes at maximum rpm. Decant into another test tube and add 4 ml of 0.1 M acetate buffer (pH 4.5) to supernatant. Mix/vortex, centrifuge at maximum rpm for 5 minutes and
decant the supernatant into another test tube. This step removes any blood fragments or foam.

- Precondition Clean Screen® extraction column with 1 x 3 ml hexane/ethyl acetate (75/25); aspirate.
- Condition Clean Screen® extraction column with the following:
  1 x 3 ml CH₃OH; aspirate.
  1 x 3 ml DI H₂O; aspirate.
  1 x 1 ml 0.1 M HCl; aspirate.
  **Note:** Use gravity flow or minimal vacuum.
- Load sample onto column at 1 ml per minute.
  **Note:** Use gravity flow or minimal vacuum.

- Wash the column with the following:
  1 x 2 ml DI H₂O; aspirate.
  1 x 2 ml 0.1 M HCl/acetonitrile (70/30); aspirate.
  Dry column with vacuum for ~5 minutes.
  1 x 200 µL hexane.
  **Note:** Use gravity flow or minimal vacuum.

- Elute THC and Carboxy THC
  1 x 3 ml hexane/ethyl acetate (75/25).
  **Note:** Use gravity flow or minimal vacuum.
- Dry Eluate. Evaporate slowly to dryness at < 40° C.
- Derivatize by adding 50 µL MSTFA (with 1% TMCS) and 50 µL of ethyl acetate. Over layer with nitrogen gas and cap. Mix/vortex. React 30 minutes at 70° C. Remove from heat source to cool.
  **Note:** Do not evaporate MSFTA solution.
- Analyze on GC/MS using an appropriate method.

2. Positive and negative controls will be utilized with all THC batch sample runs. Negative control will be commercially prepared while the positive control will be a commercially prepared whole blood base spiked with an in-house standard of THC, carboxy THC, or hydroxy THC. (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16).

**Interpretation of results:**

**Screening – GC retention time**
Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed.
Preliminary Identification – Mass Spectrum
The mass spectrum conditionally identifies the compound.

Confirmation Extraction
2. Once conditional identification has been established, a second extraction from the sample exhibit shall be performed. This extraction should confirm the results from the conditional extraction and allow the reporting of the analyte. Ideally, the second sample should be from a different sample exhibit than the first sample; however, if this is not possible, a different aliquot from the initial sample may be used. The extraction procedure may be the same as initially used (general drug extraction) or a more specialized version (e.g. opiate, benzodiazepines) as highlighted in the United Chemical Technology Manual. This extraction should also encompass the use of positive and negative controls. (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16)).

If sufficient sample is unavailable for a confirmation extraction and the preliminary ID is from Mass Spectra analysis, the analyte may be reported as a preliminary result.

- Preliminary Mass Spectra analysis of the submitted sample did indicate the presence of cannabinoids, however due to insufficient sample volume the confirmation of cannabinoids could not be reported.

If Protein Bound Drug is Suspected [by special request only or analyst discretion]

Procedure from United Chemical Technology
The following methods may be employed with whole blood, plasma or serum samples to disrupt protein binding to drug materials.

- pH modification:
  1. pH adjustment – extreme pH values (i.e. greater than 9 or less than 3). In these cases, buffer strengths greater than or equal to 0.1M should be used.
  2. pH adjustments in stages (i.e. buffering first to 5 then to 3)
- Precipitation:
  1. Employing a polar solvent (e.g. acetonitrile, acetone, or methanol). In general, two parts organic solvent to one part biological sample. Mix/vortex then centrifuge down the sample leaving the precipitate at the base of the sample vessel.
2. Inorganic salts such as ammonium sulfate or zinc sulfate may also be employed to disrupt protein binding.

- **Acid Treatment:**
  1. In this process, biological samples may be treated with formic, perchloric, or trichloroacetic acids (e.g. 50μl of 1.0M perchloric acid per 500μl of plasma or 1:1 dilution of the sample with 10% trichloroacetic acid)

- **Sonication:**
  1. The addition of an appropriate buffer (e.g. phosphate or acetate) may be followed by sonication of the sample for a period of approximately 15 minutes. The sample is then vortex mixed and centrifuged. The supernatant may be decanted off and pellet discarded.

- **Enzymatic Hydrolysis:**
  1. In this procedure, a proteolytic enzyme such as β-glucuronidase is used to disrupt the protein binding and cleave the drug. To the sample is added 1ml of an appropriate buffer for the enzyme (e.g. 1ml of a 1M acetate buffer (pH 5) with 100μl of β-glucuronidase. The sample is then heated 60°C for 1 hour. The sample is cooled, vortex mixed and centrifuged.

1. After employing one of the preceding methods, an appropriate extraction protocol may be used – such as SPE extraction (benzodiazepine or opiate), liquid/liquid extraction, etc. followed by derivatization with mstfa. Analyze on GC/MS using an appropriate method. Appropriate positive and negative controls should be used (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16)).

**Interpretation of results:**

**Screening – GC retention time**
Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed.

**Preliminary Identification – Mass Spectrum**
The mass spectrum conditionally identifies the compound.

**Confirmation Extraction**
2. Once conditional identification has been established, a second extraction from the sample exhibit shall be performed. This extraction should confirm the results from the conditional extraction and allow the reporting of the analyte. Ideally, the second sample should be from a different sample exhibit than the first sample; however, if this is not possible, a different aliquot from the initial sample may be used. The extraction procedure may be the same as initially used (general drug extraction) or a more specialized version (e.g. opiate, benzodiazepines) as highlighted in the United Chemical Technology Manual. This extraction should also encompass the use of positive and negative controls (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16)).

If sufficient sample is unavailable for a confirmation extraction and the preliminary ID is from Mass Spectra analysis, the analyte may be reported as a preliminary result.

- Preliminary Mass Spectra analysis of the submitted sample did indicate the presence of drug A, however due to insufficient sample volume the confirmation of drug could not be reported.

**Insufficient Volume: ~ 3.0 ml or less available**

Tests to be performed will change due to conditions and requirements of each case. Ensure that sufficient sample volume remains to allow for independent analysis.

Determine tests to be performed. Review the submission form for possible drug indications.

a. **Blood alcohol concentration**

If the blood alcohol concentration is > 0.08 grams/100 ml of blood, no further analysis is necessary. However, a drug screen will be performed if requested by the submitting officer in certain instances, i.e. sexual assaults, vice, burglary & theft, intoxication manslaughter, etc. If the blood alcohol concentration is < 0.08 grams/100 ml of blood, analyze for the possible presence of drugs.

b. **Reduced volume SPE for acidic/neutrals and basic drugs**

Perform analysis using the United Chemical Technologies procedure for "Forensic Sample Drug Analysis for GC or GC/MS using: Xtract® column"
XRDAH206 (large particle size and high porosity frit) or XRDAH 505.” Follow sample preparations for whole blood. **Allow gravity or positive pressure to force the sample and reagents to flow through the SPE tube.**

- Place the reconstituted residue into a crimped auto sampler vial for GC/MS analysis, using a general or toxicology method.

- Document on the data entry: method name, description of sample fractions analyzed, analysis procedure and derivatizing reagent.

- When the GC/MS is complete for Fractions A and B, **Combine both Acid/Neutral and Basic Fractions** to derivatize with MSTFA. See the Trimethyl Silyl Derivatization Procedure. **Inject the derivatized sample into the GC/MS, using the BENZOTMS or BLDDERIV-MS method.** Derivatizing the fractions will enable identification of benzodiazepines, opiates, etc. which may be undetectable when underivatized.

- Negative and positive controls will be run with all batched blood samples analyzed. (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Addition Procedure (pg. 16)).

c. **GHB Analysis**

Perform analysis by special request only. Extract ~ 0.5 ml of blood, using either method as described earlier for GHB analysis.

d. **Other Analysis**

If a specific class of drug is indicated (i.e. acidic, basic, cannabinoids, GHB, etc.), it may be necessary to dedicate the extraction for that particular drug.

**Interpretation of results:**

**Screening – GC retention time**

Retention times from the case sample must match that of the standard to within ± 0.125 minutes. A list containing retention times is maintained in the laboratory. Retention times are updated as the need arises, i.e. the capillary column is deteriorating or has been shortened or changed.

**Preliminary Identification – Mass Spectrum**

The mass spectrum conditionally identifies the compound.

**Confirmation Extraction**
1. Once conditional identification has been established, a second extraction from the sample exhibit shall be performed. This extraction should confirm the results from the conditional extraction and allow the reporting of the analyte. Ideally, the second sample should be from a different sample exhibit than the first sample; however, if this is not possible, a different aliquot from the initial sample may be used. The extraction procedure may be the same as initially used (general drug extraction) or a more specialized version (e.g., opiate, benzodiazepines) as highlighted in the United Chemical Technology Manual. This extraction should also encompass the use of positive and negative controls (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Procedure (pg. 16)).

If sufficient sample is unavailable for a confirmation extraction and the preliminary ID is from Mass Spectra analysis, the analyte may be reported as a preliminary result.

Standards and Controls

Obtain pharmaceutical standards or pure commercially prepared standards for comparison of instrumental spectra or chromatographic characteristics if the compound of interest is not available in reference libraries.

Before being placed into service, all blood controls will be verified using established lab procedures and expiration dates verified. For MSTFA derivatizing reagent – expiration date will be three years from the date of purchase. For in-house controls – three years from the date of preparation.

Check the blank result analyzed before each case sample on the GC/MS. If a blank contains presence of analytes (carryover), the case sample analysis is suspect and may require reanalysis. Blank analyses must be obtained with the same GC method as the unknown.

Additionally, carryover must be addressed in sample analysis. Carryover may occur due to the use of automated GC injection systems or due to the extreme range of drug concentration detected in toxicological samples. Regardless of cause, extreme caution is warranted when carryover is detected and requires sectional notification and review of analytical results. Appropriate action to address carryover may include reinjection with added solvent blanks between samples or reanalysis of some or all samples.

Data Analysis
Qualitative identification of an analyte is based on detection by two dissimilar methods of analysis. Gas Chromatographic retention times from the case sample must match that of the standard to within ± 0.125 minutes.

The mass spectrum of the analyte must compare to that for the standard obtained through analysis on laboratory instruments or spectra from literature or reference material. A spectrum is considered acceptable if all major ions are readily identified without the presence of any interfering ions from another analyte (i.e. co-elution). If other interfering ions are present, the analyst must consult with another section analyst to determine if analyte in question can be reported. This consultation must be documented on the worksheet. If the spectrum is not acceptable, it will not be used for confirmation. Place all spectra in the case folder.

The limit of detection for an analyte shall be defined as the concentration of the analyte required to give a signal equal to the background plus three times the standard deviation of the background.

**Reporting**

All printouts, spectra, and reports of analysis must be included in the case folder. Toxi-Lab observations must be drawn on the Toxi-Lab worksheets. Case identifiers (lab number) and analyst's handwritten initials must be present on all sheets.

If sufficient data supports identification of an analyte, it will be qualitatively listed as present. Any metabolites detected may be reported as a metabolite of a specific parent drug (e.g. codeine metabolite), or simply identified by name (e.g. α-hydroxyalprazolam, norcodeine).

An analyst who is proficient in toxicology will administratively and technically review the case folder. The presence of a checklist signifies that both administrative and technical reviews have been performed.

**Securing Evidence**

All evidence shall be sealed, dated and initialed prior to being released to the evidence section for filing and storage.

**References**

- SOFT/AAFS Forensic Toxicology Laboratory Guidelines (available on-line)
- Toxi-Lab Training Manual
United Chemical Technologies Applications Manual (UCT) (available on-line, on the Toxicology computer, and on disk)

Blood THC Liquid-Liquid Extraction

SAMPLE PREPARATION

Dilute 2 ml of blood to 8 ml with deionized water. (pH > 7.0)

EXTRACTION & HYDROLYSIS

- Extract blood with ethyl acetate/hexane (7:1)
- Make basic (~ pH 10) with 1 ml 1 N KOH (this step will hydrolyze glucuronide and extract THC)
- Organic top layer will contain Δ⁹ THC; retain in separatory funnel.
- Remove lower aqueous layer, containing CarboxyTHC
  Adjust pH to 5.0 with 1 M acetate buffer.
  Re-extract with ethyl acetate/hexane.
- Combine both organic layers together and take to dryness with nitrogen
- Appropriate positive and negative controls should be utilized along with extraction of unknown (The use of a suitable standard will be added to all samples and controls. Refer to Internal Standard Addition to Tox Samples for this info). Appropriate controls are outlined earlier in this section.

DERIVATIZATION

- Reconstitute with 10 μL ethyl acetate
- Derivatize with 10 μL MSTFA, cap and agitate.
- Heat for 15 minutes at 60 °C

ANALYSIS

- Inject ~ 1-2 μL into the GC/MS, using Cannabinoids Method
- use splitless injector sleeve
- Monitor scan for 371, 373, 488 ions
Trimethylsilyl Derivatization Procedure

Certain drugs are not readily analyzed by GC/MS. They may be too polar, thereby giving broad, ill-defined peaks. Most of these drugs may be made more stable or less polar by derivatization. The drugs that readily derivatize with silation reagent will have an acid, alcohol, or secondary amine functional group.

Sample Hydrolysis and Extraction

- Extract from matrix by the approved method for the specific drug. See specific extraction method for the drug of interest.
- Place extract into a micro reaction vial.
- Evaporate organic solvent to dryness. Do not use any type of aqueous extract, since water reacts readily with the derivatizing reagent.

Derivatization

- Reconstitute the extract in ethyl acetate (~ 50 μL for urine; 50 μL for blood)
- Add derivatizing reagent (~50-150 μL for urine; ~50 μL for blood) to the residue. Either MSTFA or BSTFA may be used.
- Place the micro reaction vial in an oven or dry block heater at 60 °C for 15 minutes. If clonazepam is suspected, heat for one hour.

Analysis

Inject 1 μL of the reaction mixture into the GC/MS, using the BENZOTMS OR BLDDERIV-MS method.
Internal Standard Procedure

Appropriate Internal Standards should be added to toxicology samples to verify completeness and precision of the various extraction methods utilized in the section. The two main extraction techniques utilized are solid phase and liquid/liquid. An aliquot of prepared ISTD should be added to all controls and case samples to verify extraction completeness. Additionally, as other components are incorporated into sample extraction, such as quantitation procedures, the ISTD method may be useful in helping verify extraction proficiency and instrumental quantitative precision.

All internal standards utilized should be verified by existing QA/QC measures before being placed into service. If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and add quantity sufficient to bring up to volume with methanol, acetonitrile, or appropriate solvent (qs). Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for three (3) years.

From the stock solutions, various working solutions may be made:

<table>
<thead>
<tr>
<th>Working solution concentration</th>
<th>amt stock used</th>
<th>volumetric flask dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>100μg/mL</td>
<td>1mL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>50μg/mL</td>
<td>500μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>20μg/mL</td>
<td>200μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>10μg/mL</td>
<td>100μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>5μg/mL</td>
<td>50μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
<tr>
<td>2μg/mL</td>
<td>20μL</td>
<td>10mL qs w/ MeOH</td>
</tr>
</tbody>
</table>

The equation to figure working solution concentration when starting with a stock solution of 1mg/mL is as follows: (amount of stock solution used in mL) / (final volume in mL) * 1000. Therefore, if 250μL of stock solution is added to 10mL volumetric flask (qs) – the resulting concentration of the solution is 0.250/10*1000 = 25μg/mL.

If different stock concentrations other than 1mg/mL are used, adjust the amount of stock used accordingly. For instance, if a concentration of stock is purchased at a level of 100μg/mL – the resulting measures take effect.
## Blood Drug Analysis Procedure

<table>
<thead>
<tr>
<th>Working solution concentration</th>
<th>amt stock used</th>
<th>volumetric flask dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>50μg/mL</td>
<td>2.5mL</td>
<td>5mL qs w/ MeOH</td>
</tr>
<tr>
<td>20μg/mL</td>
<td>1mL</td>
<td>5mL qs w/ MeOH</td>
</tr>
<tr>
<td>10μg/mL</td>
<td>500μL</td>
<td>5mL qs w/ MeOH</td>
</tr>
<tr>
<td>5μg/mL</td>
<td>250μL</td>
<td>5mL qs w/ MeOH</td>
</tr>
</tbody>
</table>

Internal standard concentrations for casework samples is therefore dependent on which working solutions the analyst will be using and the amount of specimen used from the casework sample. In other words, the amount of internal standard depends on the amount of ISTD added in μL and the concentration of the ISTD added (usually μg/mL). For instance, if 30μL of a 20μg/mL ISTD solution added to 1mL sample of a negative blood control, then a total of 0.6μg of ISTD is in the negative control (0.03 * 20 = 0.6). Since the final volume is theoretically 1mL, then this control has an ISTD concentration of 0.6μg/mL. Another example would be if 100μL of a 20μg/mL ISTD solution is added to 1mL of a negative blood control, then the total ISTD present in the sample is 2μg (0.1 * 20 = 2μg). Since the final volume 1mL, then this control has an ISTD concentration of 2μg/mL.

Therefore, starting with ISTD working solution of 5μg/mL or 10μg/mL, the following concentration levels may be attained. All of the following solutions will be in 1mL negative blood control. Remember these will be theoretical calculations only. The amount of ISTD solution should be added to an amber screw top vial and evaporated under nitrogen before the control blood is added.

<table>
<thead>
<tr>
<th>mL of ISTD control to be used</th>
<th>Concentration of ISTD in sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(5μg/mL)</td>
</tr>
<tr>
<td>1.0μg/mL also 1000ng/mL</td>
<td>200μL</td>
</tr>
<tr>
<td>0.5μg/mL also 500ng/mL</td>
<td>100μL</td>
</tr>
<tr>
<td>0.25μg/mL also 250ng/mL</td>
<td>50μL</td>
</tr>
<tr>
<td>0.10μg/mL also 100ng/mL</td>
<td>20μL</td>
</tr>
<tr>
<td>0.05μg/mL also 50ng/mL</td>
<td>10μL</td>
</tr>
<tr>
<td>0.025μg/mL also 25ng/mL</td>
<td>5μL</td>
</tr>
<tr>
<td>0.01μg/mL also 10ng/mL</td>
<td>2μL</td>
</tr>
<tr>
<td>0.005μg/mL also 5ng/mL</td>
<td>1μL</td>
</tr>
</tbody>
</table>

**PPDLAB Toxicology Casework Samples:**

Present volumes of samples used for toxicology exam include using 2mL of urine or 1 to 1.5mL of blood. Therefore, 40μL of a 5μg/mL ISTD solution added to 2.0mL of urine would yield a concentration of 0.10μg/mL or 100ng/mL and 30μL of a 5μg/mL ISTD solution added to 1.5mL of blood would yield a concentration of 0.10μg/mL or 100ng/mL. If more specialized extraction techniques are utilized such as urine
cannabinoids or benzodiazepines, the following additions should be added: For urine cannabinoids using 5mL of urine, use 100μL of a 5μg/mL ISTD solution. For urine benzodiazepines using 5mL of urine, use 100μL of a 5μg/mL ISTD solution.

### INTERNAL STANDARDS CHART TO PREPARE A WORKING CONCENTRATION OF 5μg/mL

<table>
<thead>
<tr>
<th>Extraction - SPE or liq/liq</th>
<th>ISTD used (powder/base/conc)</th>
<th>Amount stock used for 5μg/mL working</th>
<th>Volume qs</th>
<th>Manufacturer</th>
<th>Product #</th>
<th>Date rec'd</th>
<th>Lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic</td>
<td>Thiopental - 1mg/mL</td>
<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>T1019-10MG</td>
<td>6/20/2010</td>
<td>030M1651</td>
</tr>
<tr>
<td>Basic</td>
<td>Mepivacaine HCl - 1mg/mL</td>
<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>M3189-1G</td>
<td>6/20/2010</td>
<td>030M1223</td>
</tr>
<tr>
<td></td>
<td>SKF-38393 HCl - 1mg/mL</td>
<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>D047-500MG</td>
<td>10/19/2009</td>
<td>077K4626</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Prazepam - 1mg/mL</td>
<td>50μL</td>
<td>10mL</td>
<td>Sigma</td>
<td>P3654-25MG</td>
<td>6/20/2010</td>
<td>056F0684</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>11-OH Δ9-THC D3</td>
<td>500μL</td>
<td>10mL</td>
<td>Cerilliant</td>
<td>H041</td>
<td>3/26/2010</td>
<td>FE083107-02</td>
</tr>
</tbody>
</table>
Analysis for Poisons

Purpose

The analysis for poisons involves a broad range of testing procedures in order to address the myriad of compounds that could be involved. It is impossible to write a procedural approach that will include every possible poison. A systematic scheme can be used for most cases.

The most important information is a case history or profile. View the officer’s offense report and/or call and discuss the case with the investigating officer. Find out the subject’s medical history, if applicable. For example, if the patient complains of severe burning and has blackened lips, suspect a caustic substance like lye. If a subject complains of diarrhea after eating or drinking, suspect a possible laxative such as phenolphthalein. If a coffee pot smells strongly of gasoline, suspect tampering with gasoline.

Since there are so many different compounds and chemicals, this laboratory may defer analysis to another laboratory better equipped to handle some types of cases.

General Poison Scheme

1. Preparation
   Observations can reduce analysis time. The colors and odors of the unknown are useful in determining the proper course for analysis. For example, blue crystals may indicate copper sulfate, a liquid with a chlorine odor may indicate sodium hypochlorite (bleach), and a brownie that turns pink in a basic solution could have phenolphthalein mixed in it.

   A. Visual observations
      • color
      • viscosity
      • solid or liquid
      • microscopic shape (cubic, rhombic, amorphous, etc.)
      • other

   B. Olfactory observations
      • distinct odor present (pesticide odor, etc.)
      • no odor

   C. pH

   D. Solubility
Check with various liquids (water, methanol, ether, chloroform, etc.) to determine the solubility of the unknown.

**Caution:** If the unknown is suspected to be cyanide, add acid slowly in the hood.

2. **Methods to isolate unknown from the matrix:**
   
   A. Acid-steam distill
   B. Microdiffusion
   C. GC and/or GC/MS of any volatiles or organics collected
   D. Check for volatile reducing agents such as alcohols, aldehydes, acetone, sulfides. Also cyanide, phosphorus, halogenated hydrocarbons, organic nitrites.
   E. FTIR

3. **Initial tests to eliminate organic compounds (this could be performed first depending on the case history)**
   
   A. Screening Tests
   B. Toxi-Lab A extraction scheme. Sample must have a pH of ~9 (if lipids present, use the lipid procedure given in the Toxi-Lab compendium)*
   C. UV
   D. GC/MS
   E. FTIR
   F. GC-HS
   G. Flame test (for solids)
   Place a small amount of material into a small culture tube and heat directly over an open flame. If the material chars, organics could be present. If the material simply dries and turns to ash, an inorganic could be present.

   * Toxi-Lab A extraction works successfully on substances like strychnine and capsaicin (ingredient in hot peppers). Substances confirmed by GC/MS analysis.

   If all the tests above are negative, suspect possible presence of an inorganic substance, heavy metal, pesticide, etc.
4. Inorganic analysis

The following tests are a general scheme for identifying an inorganic unknown. If a particular substance is suspected, refer to the official tests for its identity listed in various books and manuals:

Analytical Procedures for Therapeutic Drug Monitoring and Emergency Toxicology (Randall C. Baselt)
Handbook of Emergency Toxicology (Sydney Kaye)
Isolation and Identification of Drugs (E.G.C. Clarke)
Merck Index
The National Formulary
Pharmacopeia of the United States
Spot Tests in Inorganic Analysis (Feigl/Anger)

Another valuable resource is through the Internet and by contact with other crime laboratories doing poison analysis.

A. Test for sulfur

1. 1 ml filtrate + 3 drops 10% freshly prepared sodium nitroprusside solution = violet color that fades away
2. 1 ml filtrate + 3 drops dilute lead acetate = black precipitate (lead sulfate)

B. Test for nitrogen

1. Gently boil 3 ml filtrate + 5 drops 10% NaOH + 5 drops dilute ferrous sulfate solution for ~0.5 minutes.
2. Add 10% HCl drop wise until brown precipitate just dissolves. Add 1 drop in excess = prussian blue precipitate (nitrogen present)

C. Test for halogens

1. When sulfur and nitrogen are both absent:
   a. Acidify 2 ml filtrate with dilute nitric acid.
   b. Add 1 ml dilute (10%) silver nitrate solution.
      
      white precipitate = chlorine
      brown precipitate = bromine
      yellow precipitate = iodine
2. When either sulfur or nitrogen is present:
   a. Add dilute sulfuric acid to filtrate until slightly acidic (filter if cloudy).
   b. To 2 ml filtrate, add 1 ml nitric acid + 1 ml of 10% silver nitrate solution = precipitate (halides present).

D. Detection of negative radicals
Using silver nitrate, nitric acid, and barium chloride:
Note: (+) = precipitate formed
(-) = no precipitate formed (or disappeared)

1. \( \text{AgNO}_3(+) + \text{HNO}_3(-) = \text{chloride, bromide, iodide,} \text{BaCl}_2(-) = \text{thiocyanate, hypochlorous acids} \)

2. \( \text{AgNO}_3(+) + \text{HNO}_3(-) = \text{nitrous acid, cyanide} \text{BaCl}_2(-) \)

3. \( \text{AgNO}_3(+) + \text{HNO}_3(-) = \text{sulfite, phosphorus, carbonic, oxalic} \text{BaCl}_2(-) + \text{HNO}_3(-) = \text{iodic, boric, selenic, telluric acids} \)

4. \( \text{AgNO}_3(+) + \text{HNO}_3(-) = \text{phosphoric, arsenic, arsenous} \text{ BaCl}_2(+) + \text{HNO}_3(-) \)

5. \( \text{AgNO}_3(-) = \text{nitrile, chloric, perchloric acids} \text{BaCl}_2(-) \)

6. \( \text{AgNO}_3(-) = \text{sulfate, fluoride} \text{BaCl}_2(-) + \text{HNO}_3(+) \)

E. Specific Screening Tests
All screening tests shall be performed using both positive and negative controls simultaneously. The screening tests listed are not complete. Refer to a reference to obtain identifying screening tests for the specific suspected unknown:

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Result</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>dilute HCl</td>
<td>+ Cl₂↑</td>
<td>ClO⁻</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>+ white ppt</td>
<td>Cl⁻</td>
</tr>
<tr>
<td>Ammonium Hydroxide</td>
<td>+ white ppt</td>
<td>Mg²⁺</td>
</tr>
<tr>
<td>Barium chloride</td>
<td>+ white ppt</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>+ white ppt</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>Schönbein test</td>
<td>+ blue</td>
<td>CN⁻</td>
</tr>
<tr>
<td>Tumeric® paper</td>
<td>+ red brown</td>
<td>B₄O₇²⁻ (borates)</td>
</tr>
</tbody>
</table>
Inorganic Acids

Acids can be analyzed with the use of silver and barium nitrate precipitation tests and by reacting with ammonium hydroxide to form ammonium salts. The salts can be isolated and identified by FTIR.

Prepare several solutions each by adding 1-2 drops of the acid to ~1 ml of deionized water. Screen for acids using silver nitrate, barium nitrate and basic barium nitrate.

1. Silver Nitrate
   Add 1-2 drops of 5% silver nitrate. If a precipitate forms, add concentrated ammonium hydroxide and observe to see if the precipitate dissolves.

2. Barium Nitrate
   Add 1-2 drops of 5% barium nitrate. Observe for precipitation formation.

3. Basic/Barium Nitrate
   Add 1-2 drops of 50% NaOH. The pH must be basic. Add more NaOH if necessary. Add 1-2 drops of 5% barium nitrate and observe for precipitation formation.

4. Ammonium salt, completely ionized
   Add 0.5 ml ammonium hydroxide to a 50 ml beaker. Slowly add 1-2 drops of the acid to the ammonium hydroxide. The pH must be >8.0. Add ~40 ml acetone to the beaker to precipitate the ammonium salt. Filter and air dry the precipitate. Analyze by FTIR.
### Results of Precipitation Tests

<table>
<thead>
<tr>
<th>Acid</th>
<th>Silver Nitrate</th>
<th>Ammonium Hydroxide</th>
<th>Barium Nitrate</th>
<th>Basic/Barium Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydriodic</td>
<td>Yellow precipitate</td>
<td>White precipitate</td>
<td>No precipitate</td>
<td>No precipitate</td>
</tr>
<tr>
<td>Hydrobromic</td>
<td>Pale yellow precipitate</td>
<td>Dissolves</td>
<td>No precipitate</td>
<td>No precipitate</td>
</tr>
<tr>
<td>Hydrochloric</td>
<td>White precipitate</td>
<td>Dissolves</td>
<td>No precipitate</td>
<td>No precipitate</td>
</tr>
<tr>
<td>Hypophosphorous</td>
<td>Black precipitate</td>
<td>No change</td>
<td>No precipitate</td>
<td>No precipitate</td>
</tr>
<tr>
<td>Sulfuric</td>
<td>No precipitate</td>
<td>N/A</td>
<td>White precipitate</td>
<td>White precipitate</td>
</tr>
<tr>
<td>Phosphoric</td>
<td>No precipitate</td>
<td>N/A</td>
<td>No precipitate</td>
<td>White precipitate</td>
</tr>
<tr>
<td>Nitric</td>
<td>No precipitate</td>
<td>N/A</td>
<td>No precipitate</td>
<td>No precipitate</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>No precipitate</td>
<td>N/A</td>
<td>No precipitate</td>
<td>No precipitate</td>
</tr>
</tbody>
</table>

**Caution:** Violent reactions may occur when mixing strong acids (particularly sulfuric acid) and strong bases. Before mixing with ammonium hydroxide, dilute the acids.

Ammonium hypophosphite and phosphate generate phosphine gas when heated. Ammonium nitrate is explosive when mixed with organic solvents and an ignition source. When making and disposing of the ammonium salts, **extreme caution should be used.**


G. **Heavy Metals**

The modified Reinsch’s test is used to identify and differentiate mercury, arsenic, antimony, and bismuth. See *Handbook of Emergency Toxicology*, page 48-54 for detailed instructions.
Add together:
1. 10-15 grams of body fluid, tissue homogenate, etc.
2. 3 ml concentrated HCl
3. small copper spiral wire or strip

Heat gently for 1-2 hours (bismuth requires 2 hours).
Examine the copper for deposition. The result must be compared to both positive and negative controls.

Deposit Color  |  Indication
---|---
silvery  |  mercury
shiny black |  bismuth
dull black |  arsenic
purple  |  antimony

A purple deposit dissolved by 10% KCN indicates selenium, tellurium or sulfur.

H. **Pesticides**
Pesticides will normally be organic in nature. If a proper case history is conducted, the pesticide in question will be limited to only a handful of possibilities. Have the investigating officer canvass the crime scene and collect all possible “poisons” related to the symptomology.

Most pesticides can be chromatographed or identified by FTIR. For those pesticides that are inorganic in nature, follow the inorganic identification scheme. Always obtain a control standard for comparison.

5. **Quality Control**
If after the preliminary testing the material is suspected of being one specific compound, it is imperative that a sample of the suspected compound be subjected to the same tests as the known to ensure accurate identification of the compound. A negative control shall also be tested.
6. **Reporting**
   Reports should follow laboratory guidelines. The format may be decided on a case by case basis. The case folder will be administratively and technically reviewed by qualified personnel.

7. **Securing Evidence**
   Evidence will be secured and handled on a case by case basis. Some samples may not be suitable for long term storage.
ETHANOL QUANTITATION PROCEDURE

Purpose

Blood and alcoholic beverages are submitted to the laboratory for determination of alcoholic content. Analysis will be performed using a capillary column headspace gas chromatography technique with n-propanol as an internal standard. Alcohol analysis using this method provides simultaneous quantitation and confirmation of alcohol. Two aliquots of samples from each specimen will be analyzed. The results of each aliquot must demonstrate acceptable precision and accuracy.

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Quality Assurance of Equipment....................................................................................................23
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ETHANOL STANDARD SOLUTIONS

1. Purpose

To establish guidelines for preparing ethanol calibrators and controls for ethanol quantitation in unknown samples. The ethanol quantitation method is based on a four-point calibration for the non-dilution method (using 0.05, 0.10, 0.20, and 0.40 grams/100 milliliters) and a six-point calibration for the dilution method (using 0.025, 0.05, 0.10, 0.20, 0.30, and 0.40 grams/100 milliliters). Additional alcohol solutions are used as controls. Analysis of purchased ethanol standard controls will verify the accuracy of the calibration, validate that the solutions were properly prepared, and are used in detector response linearity checks.

2. Solutions

- 10% Ethanol Stock Solution
- Ethanol Calibrators at various concentrations depending on the method used

3. Equipment and Supplies Needed

- Volumetric flasks
- Volumetric TD (to deliver) pipettes

4. Reagents

- Ethyl Alcohol, anhydrous, 200 proof or
- Ethyl Alcohol, 190 proof
- Sodium Chloride
- Deionized Water

5. Preparation of Solutions

Label the concentration of each solution on the flask. The preparer's initials and date must be recorded and maintained in a logbook or a file. Allow all solutions to equilibrate at room temperature before using.

10% Ethanol Stock Solution (10 grams/100 milliliters)

Dilute 12.66 ml of 200 proof ethanol (or 13.33 ml of 190 proof ethanol; or weigh 10
grams of 200 proof ethanol on the analytical balance) to 100 ml with deionized water.

**Ethanol Calibration Standards**

Ethanol calibration standards are purchased or prepared in the laboratory. Prepare the ethanol calibration standards by diluting the 10% ethanol stock solution with deionized water to 100 ml using the volumes listed in the following table:

<table>
<thead>
<tr>
<th>Ethanol Concentration</th>
<th>Volume Stock Solution/100 ml. Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025% (0.025 grams/100 ml)</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>0.05% (0.05 grams/100 ml)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>0.10% (0.10 grams/100 ml)</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>0.20% (0.20 grams/100 ml)</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.30% (0.30 grams/100 ml)</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>0.40% (0.40 grams/100 ml)</td>
<td>4.0 ml</td>
</tr>
</tbody>
</table>

**6. Quality Control**

The concentration of the solutions must be verified by analysis and compared to proven standards and/or purchased standards. Results must be within 10% of the stated value or within 0.01 gram/100 ml for standards less than 0.10%. If the results are not within these values, the standard cannot be used for casework.

- The analysis values for standards or controls above 0.10 g/100 ml should fall within the specified 10% range.
- The analysis values for standards or controls below 0.10 g/100 ml should fall within the specified 0.01 g/100ml range.
- Record the results in a logbook or a file.
- If the result is not within allowable variance of the expected value, it will be documented. Reanalyze the calibration standard curve to confirm the nature of the lack of precision. If the discrepancy is confirmed, prepare new calibration standards.

The initial verified data will be placed in the quality control file and labeled with all pertinent information including the lot number, source, date of preparation, and initials of the analyst who performed the test.
If a certificate of analysis is received for a purchased standard, place it in the quality control book.

7. **Storage, Expiration, and Disposal**

   Store solutions at room temperature or refrigerate. Shelf life is approximately one year.

   Monitor the expiration date of the purchased ethanol controls. Dispose of solutions that are expired.
n-PROPA NOL STANDARD SOLUTIONS

1. Purpose

To establish guidelines for preparation of n-propanol solutions for use as an internal standard in determining the concentration of ethanol.

2. Equipment and Supplies Needed

- Volumetric flasks
- Volumetric TD (to deliver) pipettes

3. Reagents

Although the actual concentration of the n-propanol present in the sample vials is not critical, it is imperative that the concentration remains the same for every vial throughout the entire sampling batch.

Label the concentration of each solution on the flask. The preparer’s initials and date must be recorded and maintained in a logbook or a file.

4. Preparation of Solutions

Stock Solution (10%)
Pipette 12.5 ml of N-Propanol (or weigh 10 grams of n-propanol on the analytical balance) into a 100 ml volumetric flask containing approximately 50 ml of DI water. Dilute quantum sufficient with DI water and mix thoroughly.

Working Solution (0.10%)
Pipette 1.0 ml of stock solution into a 100 ml volumetric flask containing approximately 50 ml of DI water. Dilute quantum sufficient with DI water and mix.

5. Storage, Expiration, and Disposal

Store solutions at room temperature or refrigerate. Shelf life is approximately one year. Dispose of solutions that are expired.
VOLATILE MIXTURE STANDARD SOLUTION

1. Purpose
The aqueous volatile mixture standard solution may be used in the ethanol quantitation procedure to validate chromatographically the separation of various volatile substances and potential contaminants from ethanol.

2. Solutions
Volatile mixture standard solution

3. Equipment and Supplies Needed
- Volumetric flasks
- Volumetric pipettes
- Disposable pipettes

4. Reagents
- Acetone (meets or exceeds ACS Grade)
- 95% Ethanol (meets or exceeds ACS Grade) or use ethanol stock solution
- Isopropanol (meets or exceeds ACS Grade)
- Methanol (meets or exceeds ACS Grade)
- Acetaldehyde (meets or exceeds ACS Grade)
- Deionized water

5. Preparation of Solutions
Place the following in a 100 ml volumetric flask and dilute quantum sufficient with deionized water:
- 64 µl acetone,
- 127 µl isopropanol,
- 224 µl methanol,
- 200 µl acetaldehyde,
- 133 µl 95% ethanol

The chemist may alter these levels in order to produce the best chromatograph.
6. Storage, Expiration, and Disposal

The preparer's initials and date of preparation must be recorded and maintained in a logbook or a file, or on the container. Store solutions at room temperature or refrigerate. Shelf life is determined by loss of components. Solution is quality tested in Ethanol Quantitation Procedure by Gas Chromatography.
METHOD

1. Purpose

The ethanol concentration of a biological specimen or other liquid specimen can be determined by gas chromatography headspace technique, using an internal standard. Other volatile substances can be qualitatively identified.

2. Equipment and Supplies

- Gas chromatograph with a flame ionization detector, data collection and reporting system and two capillary chromatographic columns
- Headspace auto sampler
- Crimp top clear glass headspace vials, 20 ml (23 x 75 mm)
- Crimp style caps (20 mm)
- Crimper (20 mm)
- Pipette tips, 100 µl, and seals (if necessary) for pipetter
- Single channel positive displacement or air displacement micro dispenser pipetter
- Dilutor/dispenser pipetter

3. Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample preparation should include: eye protection, lab coat, and nonporous polymer gloves (latex, nitrile, neoprene, etc.). Proper disinfecting of all contaminated surfaces is required.

Inoculation for hepatitis B is recommended for analysts who will be handling body fluid specimens.

Use appropriate safety equipment and personal protective equipment when preparing reagents and handling volatile chemicals.

4. Chemicals

- Ethanol standard solutions (prepared or purchased)
- n-propanol internal standard solution
- Volatile mixture solution
- NaCl (ACS certified or reagent grade)
5. Calibration, Standards and Controls

**Ethanol Calibration**

- Standard calibration curves are based on four-points, using 0.05, 0.10, 0.20, and 0.40 grams/100 ml or six-points, using 0.025, 0.05, 0.10, 0.20, 0.30, and 0.40 grams/100 ml.

- Calibration will be performed prior to analysis of each batch of case samples.

- The results of the linearity confirmation will be graphed as alcohol concentration versus detector response or area response ratios. The resulting graph should indicate a linear response across the range of standards tested. If the correlation coefficient \( R^2 \) is less than 0.995, the instrument should be recalibrated.

- If the analysis results of the calibrators, standards, and controls are not within the specifications listed below on "Controls", the calibration must be reanalyzed and confirmed before analyzing cases.

- If the calibration is accepted, retain a copy of the calibration graph and data in maintenance folder.

- If the instrument is shut down between sampling batches or when reanalysis of a previously tested specimen is required or a priority sample is submitted and the same analyst will perform the analysis, recalibration is not necessary if controls analyzed prior to sample analysis demonstrate linearity of the method.

**Controls**

- At least one ethanol control at a concentration to be determined by the analyst must be run. If possible, the standard should be NIST traceable.

- Analysis results must be within 10% of the target value or within 0.01 gram/100 ml for controls and standards with concentrations less than 0.10%.

- Additional ethanol controls may be analyzed at the analyst’s discretion.
Non-Ethanol Solutions

- Aqueous or blood matrix blank
  
  At least one aqueous or blood matrix blank will be analyzed at the beginning of the sample run to condition the system for the following ethanol run.

- Aqueous volatile mixture
  
  At the discretion of the analyst, a volatile mixture solution sample may be analyzed within a run sequence to demonstrate that ethanol and other volatiles are chromatographically separated and identified in an unknown mixture.

- n-Propanol internal standard
  
  An aliquot of an aqueous solution of n-propanol is added to each calibration standard, check standard, control, blank, and sample to function as an internal standard.

6. Preparation of Calibrator, Control and Specimen Sample Vials

Allow specimens, controls, standards, etc. to equilibrate to room temperature prior to sampling.

Prepare two samples for each specimen to be analyzed.

Label each 20 ml headspace vial with identifying information (i.e. case number, calibrator concentration, control concentration, etc.). Have ready for assembly caps and septa, crimper and prepared controls. **All vials must be capped and crimped immediately after addition of standards and samples. Prepare and crimp each vial individually; do not pre-prepare a set of vials with internal standard, allowing a delay in addition of sample, calibrator or control before crimping.**

Homogenizing

- Before sampling a blood specimen, determine if the vial contains anticoagulant and antibacterial agents. Record observations of any abnormality on the worksheet. Gently invert all sample vials 8-10 times 15 minutes prior to sampling to ensure proper mixing.
Observe the blood specimen for the presence of clots. If clots are present, homogenize the blood specimen with the homogenizer. Record the condition of the specimen (clotted, decomposed, etc.)

Re-cap the specimen and allow it to stand for at least 15 minutes before sampling. Bubbles present in the sample caused by the agitation of homogenization will affect analysis results.

If any clots remain, the fluid portion of the specimen may be analyzed.

**Sample Addition**

Place approximately 300 milligrams of sodium chloride (reagent grade or ACS Certified Grade) into each vial. Using the air or positive displacement pipettor, draw up slowly 200 μl of the sample. Remove any droplets hanging on the tip of the pipette before dispensing slowly into the labeled headspace vial. Do not touch the pipette tip to the sides of the vial. Dispose pipette tips and any other material (Kimwipes®, paper towels, gloves, etc.) in contact with biological fluid into the biohazard disposal container. Follow all safety precautions.

**Internal Standard Addition**

The plunger of the internal standard pipetter dispenser must be drawn up slowly. Inspect the tip of the dispenser to insure air bubbles or gaps are absent. It may be necessary to prime the plunger 2-3 times to force bubbles out of the dispenser tip. Remove any droplets clinging to the tip before dispensing into the headspace vial. Program the pipetter/dilutor to dispense 200 μl internal standard.

Alternately, an alcohol dilution method may be run which utilizes a 7-fold dilution factor:

- 150 μl of internal standard
- 150 μl of unknown/calibrator/control
- 750 μl of 1M (NH₄)₂SO₄ solution

**Crimping glass headspace vials**

Quickly cap the vial and seal by crimping. Assure uniform crimping by rotating the vial and re-crimping. Attempt to rotate the crimp cap. If the cap does not move, a good seal is present.
Pasadena police Department Regional Crime Laboratory
Standard Operating Procedures
Ethanol Quantitation Procedures

a. For calibrator samples, place into a headspace vial:
   - 300 milligrams of NaCl
   - Internal standard (200 µl)
   - Ethanol (200 µl) at concentrations of 0.05 gram/100 ml, 0.10 gram/100 ml, 0.20 gram/100 ml, and 0.40 gram/100 ml. All ethanol calibrators will be made from prepared solutions or commercially available ethanol solutions. **One vial of each concentration should be prepared during multipoint calibration.**

b. For control samples, place into a headspace vial:
   - 300 milligrams of NaCl
   - Internal standard (200 µl)
   - Ethanol standard (200 µl) at a concentration to be determined by the analyst.

c. For specimen samples (i.e. blood or alcoholic beverage), prepare two (2) headspace vials, each containing the following:
   - 300 milligrams NaCl
   - Internal standard (200 µl)
   - Specimen sample (200 µl)

7. **Alcoholic Beverage Samples**

   Dilute an alcoholic beverage sample, using the following guidelines:
   
   - If a beverage is yellowish and beer is suspected, dilute 1:50.
   - If a distilled beverage is suspected, i.e. rum, scotch, vodka, etc., dilute 1:100.
   - Use odor as a screening test for the possible type of beverage.
   - Additional dilution factors may be used as warranted.
   - Dilute the sample with deionized water in a 10 ml volumetric flask. Either 200 µl (1:50 dilution) or 100 µl (1:100 dilution) of sample is added to the volumetric flask.
   - Sample results must be multiplied by the dilution factor before reporting (e.g. for beer that is diluted 1:50, a result of 0.075 obtained will be 3.75 grams/100 ml or 3.7% w/v). The dilution factor may also be entered during the programming of the batch analysis.
8. Headspace Gas Chromatographic Analysis

Consult the instrument operations manual for proper operation of the gas chromatograph HP 7890.

The HP7890 has dual column analysis of blood alcohol concentrations. The analysis by the second column is considered a confirmatory test in the forensic community (only if the same analyte elution order on the second column is different from the first column). A GC/MS may also be run at the discretion of the analyst.

To initiate a sample run on 7890:

1. Fill in appropriate cells on sequence table (Sample type, Levels, Description, Sample amt., vial position, etc)
2. Sample amount is always 0, multiplier in most cases is 1
3. Direct data handling to the desired path
4. Save the sequence list to a unique name (ex 021408)
5. Simulate the sequence by keying simulate sequence button to verify necessary info
6. Hit start button
7. Sample queue will begin at this point

Sample load list

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone Standard</td>
</tr>
<tr>
<td>2.</td>
<td>Acetaldehyde Standard</td>
</tr>
<tr>
<td>3.</td>
<td>Calibration - low 0.05% Standard</td>
</tr>
<tr>
<td>4.</td>
<td>Calibration - medium 0.10% Standard</td>
</tr>
<tr>
<td>5.</td>
<td>Calibration - high 0.20% Standard</td>
</tr>
<tr>
<td>6.</td>
<td>Calibration - maximum 0.40% Standard</td>
</tr>
<tr>
<td>7.</td>
<td>Control Sample determined by analyst</td>
</tr>
<tr>
<td>8.</td>
<td>Blank</td>
</tr>
<tr>
<td>9.</td>
<td>Case specimen loaded with any controls inserted as deemed appropriate by the analyst</td>
</tr>
</tbody>
</table>

End - Laboratory ethanol standard determined by the analyst

- Click “load and run” on sequence screen. This will allow the analyst to save the sequence to a desired file name and begin the run.
HP G1888A Headspace Analyzer

To initiate a sample run:

1. Load vials in appropriate space

*As of January 2013, the operation of the 7890 Gas Chromatograph and G1888A Headspace Analyzer has been synched by new control software. It is now only necessary to generate a sequence on the 7890 software at which point the sequence will now control the operation of the Gas Chromatograph and Headspace Analyzer.

9. Data Analysis

Evaluation of Ethanol Control Results Analysis

Results must be within 10% of the stated value or within 0.01 gram/100 ml for controls less than 0.10% or the control cannot be used to validate casework.

If the calibration cannot be confirmed, samples must be reanalyzed.

Evaluation of Sample Results

- Sample analysis results from the duplicate samples will be added together to determine the calculated mean. The calculated mean will be truncated to three decimal places.

\[
\frac{(\text{Col A}_1 + \text{Col A}_2 + \text{Col B}_1 + \text{Col B}_2)}{4} = \text{calculated mean}
\]

- Once the calculated mean has been determined, all sample analysis results must agree within 5% of this value.

Sample analysis results \(\leq 5\%\) of the calculated mean

- If any of the sample analysis results are greater than 5% of the calculated mean, they will be deemed unacceptable and the sample will be re-analyzed. The unacceptable results will be included in the case record.

- \textbf{The calculated mean will be the used as the reported value for the sample analysis.}
Conditions for Reanalysis

- If the results do not meet the above specifications, the sample preparation and analysis may be repeated one time. Any further attempts should be investigated and discussed with other analysts.

- If the reanalysis meets the above specifications, the results will be used for reporting. If after reanalysis the results do not meet the above specification, the data and condition of the evidence must be discussed with other analysts and the Laboratory Director.

10. Analysis by GC/MS

The blood sample may be analyzed using the Agilent GC/MS if desired to provide an additional ethanol confirmation method.

- If available, the red top vacutainer should be used for this confirmation.
- If the red top vacutainer is not available, the gray top vacutainer may be used.
- If the gray top vacutainer is used, approximately one milliliter of the sample should be spun down in a high speed centrifuge.
- The resulting supernatant liquid should be used for the GC/MS confirmation.
- Prepare the sample for analysis by adding 500 μl of the n-propanol internal standard to a GC/MS analysis vial.
- Add 500 μl of the sample to the same vial.
- Cap and label the vial accordingly.
- Additional vials containing known ethanol concentrations may be prepared and processed accordingly.
- The BLDETOH Method on the Agilent is utilized for analysis of the sample. This method requires changing the injection liner and syringe to facilitate the ethanol analysis. The liner used is the HP 5183-4711 and the syringe used should be new or vacuum/heat cleaned to remove any contaminating solvents.
- Inject an air blank between each sample to ensure there is no contamination or carryover from a previous sample.
This confirmation procedure may also be used to screen for ethanol in a blood sample due to the speed of confirmation. For detailed instructions on the use of the Agilent GC/MS, refer to the Agilent section in the Controlled Substances Training Guide CS-TG 09.

11. Reporting Ethanol Concentration

*The calculated mean will be the used as the reported value for the sample analysis.*

If the result of the blood sample is greater than 0.08 g/100 ml, analysis of the specimen is complete. If the result is less than 0.08, proceed to analyze for the presence of drugs.

All alcohol results will be reported to the third decimal place.
- **Blood sample results** will be reported in units of "grams of alcohol per 100 milliliters of blood".

- If a blood sample required homogenization, the report should indicate "Due to clotting the sample required homogenization prior to analysis."

- Alcohol concentrations less than 0.01 gram shall be reported as "no alcohol detected".

- Blood alcohol evidence will be preserved for reanalysis for six months.

- If there is insufficient sample to determine the ethanol concentration (sample less than 1 ml), the report should indicate "Insufficient sample for analysis".

- If the sample is unsuitable for analysis, such as improper seal, heavily coagulated sample etc., the report should indicate "Sample unsuitable for analysis".

- If the analysis of a sample is serum (red top tube) instead of whole blood (grey top tube), a conversion factor must be applied to analysis results to reflect Texas Law. Serum and plasma ethanol concentration run 15 to 20% higher than whole blood. Accepted scientific literature supports a conversion factor of 1.15 to 1.20. By using a conversion factor of 1.2 (*Garriott’s Medicolegal Aspects of Alcohol, 5th edition*), maximum benefit is given to the suspect (i.e. serum ethanol concentration divided by 1.2 as opposed to 1.15 lowers the resulting whole blood ethanol concentration to include 95% of a population).
Alcoholic beverage samples will be reported as "% alcohol by weight" or "% w/v". It may be necessary to report the alcohol concentration as "% alcohol by volume" or "% v/v".

Conversion of alcohol concentration "by weight" to "by volume" can be accomplished with the following formula:

\[
\text{Alcohol \% v/v} = \text{Alcohol \% wt/v} \times 1.25
\]

See Appendix 1 at the end of this section for definitions as listed in the Texas Alcoholic Beverage Code.

12. Reporting Other Volatile Substances
Volatile substances may be reported only if they are detected on both sample runs.

13. Case Review
Administrative and technical reviews are required for each case. This review is documented in the LIMS and is part of the case record.
Uncertainty of Measurement

Uncertainty of Measure is a method of defining and quantifying the magnitude of the associated parameters that contribute to the inherent variance of the overall measurement process. Since all measurements have a potential for variability, determination of uncertainty as a process, attempts to allow users of such measurements to understand the reliability and suitability of the measured value.

The Toxicology section will report the uncertainty of measurements in cases where there is a “measurement that matters”. That is, where there is a reasonable expectation that a critical value or criteria may lie within the range of uncertainty for a specific measurement.

- Developing an Uncertainty Budget

The uncertainty budget for a measurement process will include both random (Type A) and systematic (Type B) uncertainties. Identified sources that can have an impact on the uncertainty measurement are:

Sample Preparation
- Homogeneity/Homogenization
- Extraction
- Derivatization
- Dilution
- Concentration

Controls
- Controls for quality assurance
  a) Reproducibility
- Uncertainty of calibrators
  a) Certified reference materials
- Uncertainty of internal standard
- Uncertainty of in-house controls
- Uncertainty of matrix of controls
- Uncertainty of reference standards

Calibration Curve
- Instrument precision
  a) Calibration Linearity

Data Processing
- Averaging of results
- Rounding and truncating
- Statistics
Analysis
- Environmental effects

Pipette
- Uncertainty of pipette used for delivery

- **Determining the uncertainty of measurement budget**
  1. Calculate the standard uncertainty based on the type of distribution the data represents
  2. Calculate the standard uncertainty subtotal: \( \sum (u_i)^2 \)
  3. Calculate the Index: Determine if the item contributes less than 1% of the standard uncertainty subtotal. Only items that contribute more than 1% will be included in calculating the total uncertainty.
  4. Calculate the Combined Standard Uncertainty: \( U_c = \text{square root of} \ (\sum (u_i)^2) \)
  5. Calculate the Expanded Combined Uncertainty using the desired coverage factor.
     i. Coverage factors: 2 will give a 95% Confidence Interval and 3 will provide a 99% confidence interval. The toxicology section will use the coverage factor of 2.

- **Blood-Alcohol uncertainty of measurement form**

**Definitions**

1. **Uncertainty of Measure** - is a parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurand.

2. **Measurement that Matters** - A determined value that is used, or may reasonably be used, by an immediate or extended customer (anyone in the Judicial process) to determine, prosecute, or defend the type or level of criminal charge(s).

3. **Type A Evaluation** – method of evaluation of uncertainty based on any valid statistical method for treating data such as calculating the standard deviation of the mean of a series of independent observations.

4. **Type B Evaluation** - method of evaluation of uncertainty by means other than the statistical analysis of a series of observations.
Pasadena police Department Regional Crime Laboratory
Standard Operating Procedures
Ethanol Quantitation Procedures

5. **Readability** - the smallest increment which the balance displays (i.e 0.01g or 0.001g)

6. **Repeatability** - closeness of the agreement between the results of successive measurements of the same item carried out under the same conditions (example: a balances ability to consistently deliver the same weight for a given mass)

7. **Linearity** – describes the difference between the observed and theoretical calibration values.

8. **Standard Uncertainty** ($u_i$) - a component of uncertainty, represented by an estimated standard deviation equal to the positive square root of the estimated variance.

9. **Distribution:**
   a. Normal : A pattern of frequency of values arrayed around a central mean value, such that the pattern is consistent with a Gaussian distribution
   b. Rectangular: A distribution of values that that there is equal probability that a value lies anywhere within the interval.

10. **Combined Standard Uncertainty** ($u_c$) - square root of the sum of the squares of the uncertainty factors, used to express the uncertainty of many measurement results.

11. **Coverage Value** ($k$) - the definition of the confidence interval; ($k = 2$ allows for a 95% confidence interval, $k = 3$ allows for a 99% confidence interval)

12. **Expanded Uncertainty** ($U$) - the interval in which a value ($y$) can be confidently asserted to lie

13. **Index** - demonstrates the individual factor’s contribution to the event uncertainty

14. **Standard Deviation** - A value associated with a normal or Gaussian distribution describing an average departure from the mean value
1. Blood Sample Collection, Preservation and Storage

**Collection**

Samples will be collected pursuant to Texas Transportation Code, §724.017. Blood Sample Guidelines.

**Preservation and Storage**

- Blood shall be collected in vials prepared with an anticoagulant and a preservative (sodium fluoride 1%). Suitable containers include commercially prepared sterile vacuum blood collection vials (gray top).

  Sodium fluoride functions as both a preservative and an anticoagulant. Examples of anticoagulants are citrate, edetate, oxalate salts and heparin.

- Additionally, Red top vials (no anticoagulant or preservative) may be collected for drug analysis.

- All blood samples received will be documented with unique identifiers (laboratory number and analyst initials). The evidence will be secured and refrigerated until analysis is complete.

2. Securing Evidence

All other evidence shall be sealed with the analyst’s initials and date placed on the seal. The evidence will be transferred to Evidence Section of the laboratory to be filed.

3. Specimens other than Blood

All other specimens and samples will be obtained and secured in cleaned vials or containers, free from alcohol or drug contamination. Preservation with an antibacterial agent is not necessary. Due to the acidic pH of urine and the high alcohol concentration of some beverages (concentrations greater than about 20% v/v), bacteria will not survive. Mold, however, will grow in beer samples. It is recommended that all alcohol analysis on beverages be performed within 24 hours of receipt to insure that alcohol
neoformation by bacteria is not a factor.

4. Final disposition after analysis

When the biological evidence is no longer required for criminal prosecution, it should be destroyed following Universal Bloodborne Pathogen Precautions.
QUALITY ASSURANCE OF EQUIPMENT

1. Purpose

To establish quality assurance guidelines for the maintenance, calibration, and repair of analytical instrumentation, balances, and pipettes.

2. Maintenance and Calibration of Laboratory Instrumentation

General Requirements for Analytical Instrumentation

All instruments will be periodically maintained and properly calibrated on the day of use in accordance with the manufacturer’s recommendations and specifications and laboratory policy. All instruments will be checked after being moved or if a major repair is performed.

Gas Chromatography

- Calibration

Gas Chromatographs must be calibrated during each analysis session. The use of additional control standards and/or frequency of recalibration may be determined by the laboratory.

The calibration and control standard charts or results will be maintained in a logbook, file, or electronically retrievable form. Calibration criteria specific to the instrument and calibration technique shall be established.

- Linearity Plots for Detectors

The linearity of each detector used must be proven across a wide range of ethanol concentrations. Standards for linearity confirmation must include the following four ethanol standards: 0.05, 0.10, 0.20, and 0.40.

The results of the linearity confirmation will be graphed as alcohol concentration versus detector response or area response ratio. The resulting graph should indicate a linear response across the range of standards tested. If the correlation coefficient ($R^2$) is less than 0.995, the issue should be investigated.
• Gas Chromatograph Maintenance

Perform regular and preventative maintenance according to the manufacturer’s recommendations or as necessary. A logbook of all maintenance will be kept with the instrument.

Gas Chromatography/Mass Spectrometry (GC/MS)

Performance Verification Check

• The Mass Selective Detector (MSD) should be tuned monthly before use or more often as needed (such as when carrier gas is changed or mass assignment fails a quality check).

• The instrument should be tuned according to the manufacturer’s instructions and must meet the manufacturer’s specification. If the manufacturer’s specifications are not met, the instrument will be removed from service until it is repaired.

• A cocaine standard should be run monthly and the scan results entered in the logbook and maintained with the tune report for that month. If the m/z ratios of 303, 182, and 82 vary by one or more, the instrument will be re-tuned and the standard re-run. If the re-tuning does not correct the discrepancy, the instrument will be removed from service until it is repaired.

• Maintain a logbook with the results.

Other GC/MS Maintenance

• Run a solvent blank before each sample run. A copy of the blank run should be maintained with the case file.

• Perform regular and preventive maintenance according to the manufacturer’s recommendations. A logbook documenting all non-routine maintenance (e.g. column replacement, filament replacement, seal replacement, vacuum oil changes, source cleaning, and major repairs) will be kept with the instrument.

Mechanical Pipette Performance Verification Check

Pipettes must be tested for accuracy and precision once a year. Calibration may be performed by an approved outside source, or may be accomplished by personnel within the laboratory, according to the laboratory’s specification.
A record of the performance verification check, calibration, and any adjustment(s) will be maintained.
REFERENCES


Personal communication with Anheuser Busch, Inc., 1999.
APPENDIX 1
TEXAS ALCOHOLIC BEVERAGE CODE DEFINITIONS

Alcoholic Beverage
Alcohol, or any beverage containing more than one-half of one percent of alcohol by volume, which is capable of use for beverage purposes, either alone or when diluted.

Ale or Malt Liquor
A malt beverage containing more than four percent of alcohol by weight.

Beer
A malt beverage containing one-half of one percent or more of alcohol by volume and not more than four percent of alcohol by weight, and does not include a beverage designated by label or otherwise by a name other than beer.

Distilled Spirits
Alcohol, spirits of wine, whiskey, rum, brandy, gin, or any liquor produced in whole or in part by the process of distillation, including all dilutions or mixtures of them, and includes spirit coolers that may have an alcoholic content as low as four percent alcohol by volume and that contain plain, sparkling, or carbonated water and may also contain one or more natural or artificial blending or flavoring ingredients.

Liquor
Any alcoholic beverage containing alcohol in excess of four percent by weight, unless otherwise indicated. Proof that an alcoholic beverage is alcohol, spirits of wine, whiskey, liquor, wine, brandy, gin, rum, ale, malt liquor, tequila, mescal, habanero, or barreteago, is prima facie evidence that it is liquor.

Mixed Beverage
One or more servings of a beverage composed in whole or part of an alcoholic beverage in a sealed or unsealed container of any legal size for consumption on the premises where served or sold by the holder of a mixed beverage permit, the holder of a daily temporary mixed beverage permit, the holder of a caterer's permit, the holder of a mixed beverage late hours permit, the holder of a private club registration permit, or the holder of a private club late hours permit.

Wine and Vinous Liquor
The product obtained from the alcoholic fermentation of juice of sound ripe grapes, fruits, berries, or honey, and includes wine coolers.
Wine Cooler

An alcoholic beverage consisting of vinous liquor plus plain, sparkling, or carbonated water and which may also contain one or more natural or artificial blending or flavoring ingredients. A wine cooler may have an alcohol content as low as one-half of one percent by volume.
QUALITY ASSURANCE

Purpose
The laboratory is responsible for assuring the integrity of the data, results, and interpretations resulting from the analyses performed. A quality assurance program includes quality control, training, continuing education, proficiency testing, and quality assessment (technical review and audits.)

QUALITY CONTROL
Quality control addresses the use of standards, controls and calibrations.

Chemical Standards and Reagents
Pure chemical standards should be obtained for parent drugs and metabolites. Each will be documented with the date opened. Each will be verified and stored appropriately to preserve its integrity. Compare all standard chromatographic and spectral data against any references to ensure accuracy of the standard obtained. Dated standards shall be destroyed if they are expired or retained for training purposes only.

- All prepared stock and working solutions will be labeled, dated and initialed by the preparer.
- Reagents should be checked periodically with a known standard to ensure integrity.
- Each batch of immunoassay control panels will be checked with positive and negative controls before use.
- Toxi-Lab® reagents are checked with standards and documented in the case notes for each sample run.
- Acids, bases, and buffers are pH checked at the time of preparation.

No reagent or other chemical preparation will be used in casework if it is not working properly or if it is contaminated. If an analyst has reason to suspect that a reagent or other chemical preparation is not working properly or is contaminated, he or she must:

- Check the reagent or system with standards or proper sample controls.
- Discard the reagent if it fails the quality check, prepare a new reagent, and quality check the reagent with a known standard.
- Cease performing casework with these reagents until the problem has been corrected.
- Identify casework that may have been affected by the reagents/chemicals that failed the quality check and re-test with quality checked reagents.
Instruments and Calibrations

Instruments will be maintained periodically. Quantitative instruments should be calibrated when a batch of samples will be analyzed. Document all preventive maintenance performed, by whom and the dates in an instrument logbook. The actions taken for routine periodic instrument performance checks and calibrations should also be recorded in the instrument log.

For quality control information on the GC headspace analyzer, refer to the section on ethanol quantitation.

Gas Chromatography/Mass Spectrometry (GC/MS) plus TSD Detector

Performance Verification Check

- The Mass Selective Detector (MSD) should be tuned monthly before use or more often as needed (such as when carrier gas is changed or mass assignment fails a quality check).
- The instrument should be tuned according to the manufacturer’s instructions and must meet the manufacturer’s specifications. If the manufacturer’s specifications are not met, the instrument will be removed from service until it can be repaired.
- A cocaine standard should be run monthly and the scan results entered in the logbook and maintained with the tune report for that month. If the m/z ratios of 303, 182, and 82 vary by one or more, the instrument will be re-tuned and the standard re-run. If the re-tuning does not correct the discrepancy, the instrument will be removed from service until it can be repaired.
- The retention time for the cocaine standard will be documented for the TSD detector.
- Maintain a logbook with the results.

Other GC/MS Maintenance

- Run a solvent blank before each sample run. A copy of the blank run should be maintained with the case file.
- Perform regular and preventive maintenance according to the manufacturer’s recommendations.
- A logbook documenting all non-routine maintenance (e.g. column replacement, filament replacement, seal replacement, vacuum oil changes, source cleaning, and major repairs) will be kept with the instrument.
Malfunction of an Instrument or Balance

- If an instrument or balance fails the performance check or a performance problem is detected during routine maintenance, it must be removed from service, the team leader or Quality Manager must be notified and the problem recorded in the logbook.
- No instrument or balance is to be used if it is not in proper working order.
- Repair or have the instrument or balance repaired and perform routine quality control procedures with standards to ensure it is working properly before it is returned to service.
- Verification with standards will be performed after routine maintenance if the performance of an instrument could be affected.
- Keep a record of all repairs and routine maintenance in an appropriate logbook.

Method Development and Validation

A new method may be incorporated if it has greater sensitivity, it is more efficient and/or economical and it produces cleaner results. New methodology will be incorporated into the toxicology protocol when it has been established that the method is accepted in the forensic scientific community. Acceptance of the method is understood when the method is obtained from a peer-reviewed publication or received from other scientists in the same discipline.

The method must be tested with known controls before using on unknown samples from casework. It shall be subjected to a thorough evaluation to assure its validity. The new method’s accuracy and precision must be proven to be equal or greater than the existing method, before the new method can be implemented. The method must be reproducible, as demonstrated by multiple analyses. The new procedure will be evaluated on its shortcomings and strengths. After thorough testing, the method will be judged by the analyst and the Quality Assurance Manager before replacement of the existing method.

Methods utilized by the laboratory are based on the following:
- Acceptance within the forensic science community
- Reproducible accurate results
- Validated methods
- Available laboratory equipment, supplies and reagents

TRAINING

Training can be formal coursework taught by an outside agency or informal classes taught by other analysts. Examples of training include:
• Courses in theory, analytical techniques and practical application
• In-house training in procedures and protocols
• Reading of reference materials

CONTINUING EDUCATION
Continuing education is provided through the following examples:
• In-house training
• Keeping abreast with scientific developments
• Reading scientific literature
• Discussions with colleagues
• Attendance at professional conferences
• Workshops provided by professional groups
• Workshops in analytical techniques, interpretations, and practical applications
• College or university courses

PROFICIENCY TESTING
Assurance of accurate and precise conclusions can be accomplished by participating in internal and external open and blind proficiency examinations. A proficiency test will be completed each year by any analyst performing casework analysis.

External open blind proficiency program
A proficiency service is presently being subscribed for the following areas:
• Toxicology
  CAP (College of American Pathologists) Forensic Sciences
  Quality Forensics
• Blood Alcohol
  Collaborative Testing Services Inc., P.O. Box 650820, 21331 Gentry Drive, Sterling, VA 20165
  United States Department of Transportation, Research and Special Programs, Administration, Safety and Environmental Technology Division, Kendall Square, Cambridge, Massachusetts 02142

Internal open proficiency program
A urine specimen may be submitted from an individual who is using prescribed medication. The time of collection, time of the last dose of medication, and the list of substances ingested are documented. Analysis will proceed as for casework on unknowns.
Other proficiency samples, such as past proficiency samples and previous casework that has been adjudicated, may also be used as internal open blind proficiencies.

**QUALITY ASSESSMENT**

Quality assessment monitors the validity of methods and specific results through technical review, audit, etc. A quality assessment program provides the documentation for management to demonstrate competence and to address complaints.

**Technical Review**

All case notes and reports will be reviewed by a qualified analyst. The technical review shall monitor analysis methods, calculations, and scientific conclusions on the worksheet and proper reporting on the supplementary report. Laboratory numbers and the chemist's initials must be on every page of the examination sheets and spectra.

Analysis of all unknowns must be performed with the proper controls and calibrators. The review must determine that the minimum appropriate analyses have been performed to accept the scientific conclusion. The chromatographic or spectral analysis should conform to a standard in retention time, conformity, proportion, and peak intensities. There shall be sufficient resolution in the chromatograph analysis to compare to a control.

Toxicology samples shall have a minimum of two identifying tests. Each positive result should be confirmed with a separate aliquot from the original or a second sample by a different analytical technique. Exceptions may be made when the analytical technique is simple and specific (i.e. alcohol analysis and quantitation by gas chromatography) or when a different analytical technique is not available.

**Audits**

An audit is an evaluation process to determine the degree of adherence to prescribed norms (criteria, standards) resulting in an opinion or judgment. Audits on random case folders and evidence should be conducted periodically. The audit should evaluate case folders and evidence storage, the accessibility of both, and documentation of chain of custody identifying evidence location.

The audit should examine each case folder and evidence for complete labeling of every page in the folder and the evidence (i.e. laboratory number and initials). In addition, the audit will look at documentation of chain of custody, the completeness of the folder (whether pages, such as graphs, worksheets, or supplements, etc., are missing), and the presence of a technical review.

**PPDLAB Toxicology Section Quality Check Procedures**

Urine Drug quality checks to be run with all batched urine specimens analyzed:
All reagents and consumables that would be used in a PPDLAB urine drug analysis are utilized with a positive and negative control run with casework samples. The control urine is obtained from BioRad in the following levels:

- BioRad Liquichek Urine Toxicology – Negative Control
- BioRad Liquichek Urine Toxicology – Level C2
- BioRad Liquichek Urine Toxicology – Level C4

The BioRad Negative Control will comprise the negative control while either BioRad Level C2 or C4 will comprise the positive control. The major analytes in the BioRad Controls are as follows:

<table>
<thead>
<tr>
<th>MDA</th>
<th>MDMA</th>
<th>MDEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Amphetamine</td>
<td>d-Methamphetamine</td>
<td>Amobarbital</td>
</tr>
<tr>
<td>Butalbital</td>
<td>Pentobarbital</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>ë-hydroxyalprazolam</td>
<td>Nordiazepam</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>11-Nor-ë-9-THC-9-COOH</td>
<td>Morphone-3-ë-D-glucuronide</td>
</tr>
<tr>
<td>Methadone</td>
<td>Methaqualone</td>
<td>Codeine</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>Norpropoxyphene</td>
<td></td>
</tr>
</tbody>
</table>

Additional levels are available from BioRad which may be utilized in the QC assay; however, these three levels have proven ideal in establishing limits of detection and instrument response.

Consumables used include solid phase extraction cartridges, toxi grams a and b, InstantView immunoassay kit, Triage immunoassay kit, test tubes, vial inserts, amber vials, concentration cups, disposable beakers and pipettes. Reagents include deionized water, toxi tubes a and b, phosphate buffers, acetic acid, methanol, ethyl acetate, methylene chloride, ammonium hydroxide, hexane, β-glucuronidase, sodium hydroxide, isopropanol, and derivatization reagent (MSTFA AND BSTFA). Before new consumables and reagents are introduced into casework samples; item description, lot numbers, expiration dates and dates introduced into analysis should be documented.

Basic extraction scheme follows PPDLAB Toxicology section protocols for urine drug analysis. Solid phase extraction is used for confirmation via GC/MS analysis. Toxilab analysis is used as a secondary ID method while the immunoassay serves as the initial screen. Additionally, the TSD detector may be utilized as a secondary testing mechanism.

Analysis of the negative urine control should yield no drugs present on both immunoassay kits. Toxilab screening should also reveal a negative response.
Additionally, any extraction procedure and its related instrumental analysis method should yield a negative response. Analysis of the positive urine control (either Level C2 or C4) should identify all the previously mentioned analytes on the general extraction schemes or specialized extraction schemes (i.e. THC, benzodiazepines, etc.) and its related instrumental analysis method. Immunoassays of the Level C4 control should yield a positive response on all drug classes while the Level C2 control may only yield positive results for several drug classes based on that kit’s cut-off points. Toxilab screening response is dependent on which positive control level is used. Most but not all analytes should indicate a positive response on Toxilab. Additionally, the TSD detector on the Varian 4000 may be utilized as a secondary testing mechanism on urine specimens; therefore, the response of the TSD detector will be evaluated on all control samples.

All results will be documented and maintained in a Toxicology Quality Check binder

**Blood Drug quality check to be run with all blood casework samples:**

All reagents and consumables that would be used in a PPDLAB blood drug analysis are utilized in a quality control assays. Control Blood is obtained from Clinical Controls or made in-house.

The analytes in the positive control stock solution may include but are not limited to the following drugs:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Level</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(3-chlorophenyl)piperazine - mCPP</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>11 nor d9 carboxy THC</td>
<td>-</td>
<td>unknown</td>
</tr>
<tr>
<td>11-OH d9 THC</td>
<td>-</td>
<td>unknown</td>
</tr>
<tr>
<td>alpha-OH alprazolam</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Benzoylcegonine</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Butalbital</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Dextropropoxyphene</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Ecgonine methyl ester</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>-</td>
<td>unknown</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>MDA</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Nordiazepam</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td>Morphine-β-3D glucuronide</td>
<td>-</td>
<td>0.02 mg/ml</td>
</tr>
</tbody>
</table>
PCP - 0.02 mg/ml
Quetiapine - unknown

The negative blood utilized will be the Clinical Control Whole Blood Ethanol or the Whole Blood Volatile Control. These controls are used for the Lab’s blood alcohol verification methods and contain only volatile components.

Consumables used include solid phase extraction cartridges, test tubes, vial inserts, amber vials, concentration cups, disposable beakers and pipettes. Reagents include deionized water, phosphate buffers, acetic acid, methanol, ethyl acetate, methylene chloride, ammonium hydroxide, hexane, sodium hydroxide, isopropanol, and derivatization reagent (MSTFA or BSTFA). Before new consumables and reagents are introduced into casework samples; item description, lot numbers, expiration dates and dates introduced into analysis should be documented. For MSTFA derivatizing reagent, the expiration date will be three years from the date of purchase. For in-house controls, the expiration date will be three years from the date of preparation.

Basic extraction scheme follows PPDLAB Toxicology section protocols for blood drug analysis. A negative and positive control will be run with all batched blood casework samples. It is expected that following section protocols, all analytes in the positive control should be detected on GC/MS confirmation. Analysis of the negative control should yield no drugs present on any extraction procedure and its related instrumental method. Additionally, the TSD detector on the Varian 4000 may be utilized as a screening mechanism on blood samples; therefore, the response of the TSD detector will be evaluated on all control samples.

All results will be documented and maintained in a Toxicology Quality Check binder. All lot numbers and expiration dates of controls, consumables and reagents will also be documented in this binder.

Blood Alcohol quality check to be run each quarter:

All reagents and consumables that would be used in a PPDLAB blood alcohol analysis are utilized in a quality control assays. The whole blood ethanol control and whole blood volatile control is obtained from Clinical Controls. Aqueous ethanol standards will be NIST traceable or actual NIST ethanol standards.

Additionally, ethanol and n-propanol stocks will be reformulated each quarter. After 24 hour equilibration, n-propanol and varying ethanol working solutions will be made. These solutions will be allowed to equilibrate for 24 hours before use in any quantitation analysis. Also, PPDLAB volatile standards will be reformulated each quarter and allowed to equilibrate 24 hours before use in any quantitation analysis.
The solutions to be reformulated each quarter include:

- n-propanol stock solution
- 0.100 g/dl n-propanol
- 0.050 g/dl EtOH solution
- 0.100 g/dl EtOH solution
- 0.200 g/dl EtOH solution
- 0.400 g/dl EtOH solution
- PPDLAB Volatile Mix 1 (EtOH, acetone, acetaldehyde, MeOH, and IsoP)
- ethanol stock solution
- 0.040 g/dl EtOH solution
- 0.080 g/dl EtOH solution
- 0.160 g/dl EtOH solution
- 0.320 g/dl EtOH solution
- 0.640 g/dl EtOH solution

Consumables used include headspace vials, crimp caps, disposable beakers and pipettes. Reagents include deionized water, sodium chloride, ammonium sulfate, ethanol, n-propanol, methanol, isopropanol, acetone, acetaldehyde, chloroform, toluene, and formaldehyde.

Basic analytical scheme follows PPDLAB Toxicology section protocols for blood alcohol analysis. Each new lot of consumables and reagents received by the Lab are included in the quarterly check to ensure utmost confidence in analysis procedures.

Additionally, each time a new control lot of whole blood ethanol or whole blood volatile is received, a GC quantitation run consisting of at least 15 samples will be performed. This run will establish a mean value for each of the controls. Before new consumables and reagents are introduced into casework samples; item description, lot numbers, expiration dates and dates introduced into analysis should be documented.
ABBREVIATIONS AND DEFINITIONS

SCOPE
To provide a list of useful abbreviations and definitions

ABBREVIATIONS

+/−................................................plus or minus
AB .................................................. analytical balance
AEM ................................................ anhydrous ecgonine methyl ester
AM .................................................. used for some synthetic cannabinoids
amph or amp .................................. amphetamine
APAP ............................................. acetaminophen
? .................................................... indication
1,4-BD .......................................... 1,4-butanediol
BAR .............................................. barbiturate
BB .................................................. bulk balance
BE .................................................. benzoylcegonine
BH .................................................. biohazard
BSTFA .......................................... derivatizing agent (TMS derivatives)
BZD .............................................. benzodiazepine
BZO .............................................. benzodiazepines
BZP .............................................. benzylpiperazine
c or c/ .......................................... containing
cig ................................................ cigarette
cig stub ........................................ cigarette stub
coc ................................................ cocaine
COC ............................................ drug screening strip = cocaine metabolite i.e. BE
CP ................................................ synthetic cannabinoids (Charles Pfizer)
CS..............................controlled substance
DD..............................dangerous drug
D.O............................destroy
DHC ............................dihydrocodeine
EA .............................ethyl acetate
EDDP ..........................methadone metabolite
EDIA.............................Evidence Destroyed in Analysis
EME ...........................ecgonine methyl ester
ENV ............................envelope
EtAc............................ethyl acetate
EtOH...........................ethanol
EV ..............................evidence
g.............................grams
GBL.............................γ-butyrolactone
GC .............................gas chromatograph
GHB ...........................γ-hydroxybutyric acid (γ-hydroxybutyrate)
HC .............................hydrocodone
HM .............................hydromorphone
HSB ...........................heat sealed bag
HU..............................synthetic cannabinoids (Hebrew University)
Ind............................indication
JWH............................synthetic cannabinoids after James W. Hoffman
kg.............................kilograms
lb.............................pounds
L...............................liters
LSD...........................lysergic acid diethylamide
Mari............................marihuana
mCPP ........................................................ meta-chlorophenylpiperazine
MDA .......................................................... 3,4-methylenedioxy amphetamine
MDE .......................................................... 3,4-methylenedioxy ethylamphetamine
MDMA ......................................................... 3,4-methylenedioxy methamphetamine
MDPV ........................................................ methylenedioxypyrovalerone
MeCl .......................................................... methylene chloride
MeOH ........................................................ methanol
metb or mtb ................................................ metabolite
meth or MET ................................................ methamphetamine
mg .......................................................... milligrams
MS .......................................................... mass spectrometer
MSTFA ........................................................ derivatizing agent (TMS derivatives)
MTD ........................................................ methadone metabolite i.e. EDDP
neg .......................................................... negative
nor-HC or NHC ........................................... norhydrocodone
OPI .......................................................... opiates
oz .......................................................... ounces
PCP .......................................................... phencyclidine
pkg .......................................................... package
pos .......................................................... positive
PS .......................................................... plant substance
Rf .......................................................... retention factor (TLC)
RT .......................................................... retention time
TFMPP ........................................................ trifluoromethylphenylpiperazine
THC ........................................................ tetrahydrocannabinol
THCA ........................................................ carboxy-THC
TLC .......................................................... thin layer chromatography
DEFINITIONS

- **Alkaloid** - Group of basic, nitrogenous plant products which have marked physiological action. The majority of these are complex heterocyclic compounds.

- **Addiction** - Drug addiction is a state of periodic or chronic intoxication produced by the repeated consumption of a drug. Its characteristics include:
  - An overpowering desire or need (compulsion) to continue taking the drug and to obtain it by any means;
  - A tendency to increase the dose;
  - A psychic (psychological) and generally a physical dependence on the effects of the drug;
  - An effect detrimental to the individual and to society.

- **Adulterant** - Compounds which are added either to attempt to increase or enhance the effect of a controlled substance on the body, or to fool the pusher and/or addicts into thinking they have better "stuff" than they have actually purchased.

- **Analgesic** - Insensibility to pain without loss of consciousness; pain killer.

- **Anesthetic** - Causes loss of sensation with or without loss of consciousness.

- **Anorectic** - Causes loss of appetite.

- **By-Products** - Compounds found in addition to the compound of interest after a chemical reaction or an extraction.

- **Central Nervous System** - The brain and spinal cord.

- **Controlled Substance** - A substance, including a drug and an immediate precursor, listed in Schedules I through V or Penalty Groups 1 through 4.
Criminalistics - That science which involves the application of the physical sciences (e.g., chemistry, physics, biology) to the investigation of crime.

Dangerous Drug - A device or a drug that is unsafe for self-medication and that is not included in Schedules I through V or Penalty Groups 1 through 4 of Chapter 481 in the Texas Controlled Substances Act. The term includes a device or a drug that bears or is required to bear the legend:
A. Caution: federal law prohibits dispensing without prescription; or
B. Caution: federal law restricts this drug by or on the order of a licensed veterinarian.

Dependence - Drug dependence is a state of psychological or physical dependence or both, which results from chronic, periodic, or continuous use of a drug.

Depressant - Any of several drugs which sedate by acting on the central nervous system. Medical uses include the treatment of anxiety, tension, and high blood pressure.

Diluent (Dilutants) - Diluents are compounds added strictly for "bulk" to increase the quantity of a controlled substance for distribution.

Drug - A substance, other than a device or a component, part, or accessory of a device, that is:
- recognized as a drug in the official United States Pharmacopoeia, official Homeopathic Pharmacopoeia of the United States, official National Formulary, or a supplement to either pharmacopoeia or the formulary;
- intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or animals;
- intended to affect the structure or function of the body of man or animals but is not a food; or
- intended for use as a component of a substance described by Paragraph A, B, or C (from Texas Controlled Substances Act).

Felony - A grave crime declared to be a felony by the common law or by statute regardless of the punishment actually imposed.
• **Forensic Science** - Forensic science is a broad term denoting the application of medical, social, behavioral, and other sciences to the administration of justice.

• **Habituation** - A condition, resulting from the repeated administration of a drug, which includes these characteristics:
  
  - a desire (but not a compulsion) to continue taking the drug for the sense of improved well-being that it engenders;
  - little or no tendency to increase the dose;
  
  - some degree of psychic dependence on the effect of the drug but absence of physical dependence and, hence, no abstinence syndrome;
  - a detrimental effect, if any, primarily on the individual.

Simplified, habituation is the psychological desire to repeat the use of a drug intermittently or continuously because of emotional reasons.

• **Hallucinogen** - Both natural and synthetic hallucinogens are substances that distort the perception of objective reality. They induce a state of excitation of the central nervous system. A person using hallucinogens will be disoriented, have delusions, and hallucinations.

• **Hypnotic** - An agent that induces sleep.

• **Impeach** - To discredit a witness.

• **Local Anesthetic** - Causes a numbing effect.

• **Marihuana** - The plant *Cannabis sativa L.*, whether growing or not, the seeds of that plant, and every compound, manufacture, salt, derivative, mixture, or preparation of that plant or its seeds. The term does not include:
  
  - the resin extracted from a part of the plant or compound, manufacture, salt, derivative, mixture, or preparation of the resin;
  - the mature stalks of the plant or fiber produced from the stalks;
  - oil or cake made from the seeds of the plant;
  - a compound, manufacture, salt, derivative, mixture, or preparation of the
mature stalks, fiber, oil, or cake; or
- the sterilized seeds of the plant that are incapable of germination.

- **Misdemeanor** - A crime less serious than a felony. For possession less than 28 grams, substances in Penalty Groups 3 and 4. For possession marihuana, less than 4 ounces. For possession without a prescription, all dangerous drugs.

- **Narcotic** - Something that soothes, relieves or lulls; drug that in moderate doses dulls the senses, relieves pain, and induces profound sleep but in excessive doses causes stupor, coma, or convulsions (dictionary definition). This term in its medical meaning refers to opium and opium derivatives or synthetic substitutes. They are the most effective agents for the relief of pain and are central nervous system (CNS) depressants.

- **Narcotic Drug** - Means any of the following, produced directly or indirectly by extraction from substances of vegetable origin, independently by means of chemical synthesis, or by a combination of extraction and chemical synthesis:
  1. opium and opiates, and a salt, compound, derivative, or preparation of opium or opiates;
  2. a salt, compound, isomer, derivative, or preparation of a salt, compound, isomer, or derivative that is chemically equivalent or identical to a substance listed in the first bullet other than the isoquinoline alkaloids of opium;
  3. opium poppy and poppy straw; or
  4. cocaine, including:
     a. its salts, its optical, position, or geometric isomers, and the salts of those isomers;
     b. coca leaves and a salt, compound, derivative, or preparation of coca leaves; and
     c. a salt, compound, derivative, or preparation of a salt, compound, or derivative that is chemically equivalent or identical to a substance described by bullets one or two, other than decocanized coca leaves or extractions of coca leaves that do not contain cocaine or ecgonine (from Texas Controlled Substances Act).

- **Opiate** - A substance that has an addiction-forming or addiction-sustaining liability similar to morphine or is capable of conversion into a drug having addiction-forming or addiction-sustaining liability. The term includes its racemic and levorotatory forms. The term does not include, unless specifically designated as controlled under Section 481.038, the dextrorotatory isomer of dextromethorphan (from Texas
Controlled Substances Act).

- **Over ruled** - When an objection is over ruled you can answer the question.

- **Perjury** - Act of willfully swearing or testifying falsely.

- **Physical Dependence** - An adaptive state caused by repeated drug use that reveals itself by development of intense physical symptoms (withdrawal syndrome) when use of the drug is stopped.

- **Potentiation** - Occurs when the combined action of two or more drugs is greater than the sum of the effects of each drug taken alone. Potentiation can be very useful in certain medical procedures. For example, physicians can induce and maintain a specific degree of anesthesia by using another drug to potentiate the primary anesthetic agent. Potentiation may also be dangerous. For example, barbiturates and many tranquilizers potentiate the depressant effects of alcohol.

- **Psychological Dependence** - An attachment to drug use which arises from a drug's ability to satisfy some emotional or personality need of an individual. This attachment does not require a physical dependence, although physical dependence may seem to reinforce psychological dependence.

- **Sedative** - An agent that quiets or calms activity.

- **Stimulant** - Any of several drugs which act on the central nervous system producing excitation, alertness and wakefulness. Medical uses include the treatment of mild depressive states, overweight and narcolepsy, a disease characterized by an almost overwhelming desire to sleep.

- **Sustained** - When an objection is sustained, you cannot answer the question.

- **Tolerance** - With many drugs, a person must keep increasing the dosage to maintain the same effect.

- **Voir Dire** - Preliminary examination of witness (e.g., criminalist) in order to determine qualifications and competency, or to examine in depth the chain of custody of the evidence.
SIMPLIFIED COURT DEFINITIONS

These are some suggested short definitions that could be useful when testifying about certain testing procedures.

- **SPOT TESTS**
  Series of screening tests which indicate what type of compounds might be present.

- **MICROCRYSTALLINE TEST**
  An unknown sample is dissolved in a reagent and viewed under the microscope for the formation of characteristic crystals.

- **THIN LAYER CHROMATOGRAPHY**
  Comparison of adsorption rates of a known and an unknown sample in a solvent system on a silica gel surface.

- **GAS CHROMATOGRAPH**
  An instrument which identifies the components of a mixture based upon their retention times on a separating column.

- **GAS CHROMATOGRAPH QUANTITATION**
  A quantitation based on the comparison of the areas under the peaks for an unknown sample and a standard.

- **ULTRAVIOLET SPECTROPHOTOMETER**
  An instrument which identifies a sample based on its light absorption pattern. Ultraviolet light at different wavelengths passes through the sample and a graph is produced showing where the light is absorbed. Starting with a known sample weight and a known solvent volume, the percentage of purity can be determined.

- **GAS CHROMATOGRAPH/MASS SPECTROMETER**
  An instrument which separates and specifically identifies the components of a mixture based upon their masses.

- **FOURIER TRANSFORM INFRARED SPECTROPHOTOMETRY**
  An instrumental technique which identifies substances based upon their unique infrared light absorption patterns.
ADULTERANTS
Adulterants are compounds which are added either to attempt to increase or enhance the effect of a controlled substance on the body, or to fool the pusher and/or addicts into thinking they have better "stuff" than they do.

DILUENTS (DILUTANTS)
Diluents are compounds added strictly for "bulk" to increase the quantity of a controlled substance for distribution. The lawyers all use the word “dilutants.”

BY-PRODUCTS
Compounds found in addition to the compound of interest after a chemical reaction or an extraction.

INSTRUMENT
A device that uses scientific principles to make accurate, precise, and repeatable measurements.

MACHINE
A device that converts energy into motion or motion into energy.
TOXICOLOGY SECTION TRAINING OUTLINE

PURPOSE

The outline provides a survey of the training program and the expectations of the trainee during the training period. An approximate period of time is provided for each category of training. Time allotted per training period may vary.

ORIENTATION (one week)

The trainee will be given a short period for familiarization of the overall function of the laboratory.

GENERAL INFORMATION (two to four weeks)

The trainee will be expected to complete the reading assignments and receive general instruction in the day to day activities of the Toxicology section.

- Reading material
- Introduction to Toxicology
- Introduction to Centralized Evidence Receiving
- Case and Evidence Documentation
- Instrumentation
- Non-instrumental techniques
- Introduction to court procedures and legal aspects
- Projects

TOXICOLOGY ANALYSIS TRAINING (one to six months)

The trainee will be given analysis training in the various areas of the toxicology section listed:

- Biological Fluids (urine, blood and stomach contents)
- Tablets and capsules
- Powders
- Liquids
- Miscellaneous materials
- Quality control and assurance
- Examination documentation and report writing
- Oral and written examinations
APPLICATIONS OF TOXICOLOGY ANALYSIS TECHNIQUES (one to six months)

Training in analysis of toxicology samples will progress from standard samples to previously analyzed samples followed by competency testing with proficiency samples. Samples used in training include:

- Known samples using older proficiency urine specimens.
- Known blood alcohol samples prepared internally.
- Previously analyzed samples of casework with direct supervision.
- Competency testing for blood alcohol and urine drugs.

COURT TESTIMONY

- Training - Observation of other analysts and discussion
- Mock Trial – testimony given in a courtroom with videotaping of testimony, when available, and follow-up critique

BREATH ALCOHOL TECHNICAL SUPERVISOR TRAINING

If the trainee’s duties are to include work as a Technical Supervisor, follow the regulations established by the Office of the Scientific Director with the Texas Department of Public Safety.

EXAMINATIONS

Oral, written and practical laboratory examinations will be given periodically until training is completed. Examinations may also be given unannounced. Discussions and evaluation of the examinations will provide an assessment of the employee’s progress during training.

OUTSIDE TRAINING

The trainee will be provided an opportunity to attend available outside instruction through other agencies.
TOXICOLOGY SECTION TRAINING GUIDE

ORIENTATION

1. The laboratory will ensure that the new employee has received the various items:
   - Identification Badge
   - Safety equipment, work items, work area assignment, lab coats etc.
   - Training Verification Book (employee will keep all certificates for training and a copy of their resume)

2. The new employee will become familiar with the operations and responsibilities of the various sections within the laboratory. The individual may visit the various sections to talk to the personnel and get acquainted with the various types of analysis, evidence, reports, files etc.

3. The individual will be provided with and will read and understand the following:
   - Laboratory Operations Guide
   - The Laboratory Safety Manual
   - Any information provided by Human Resources

4. The employee will become familiar with the Operational Guide and Analytical Procedures for the toxicology section.

5. The employee will attend any scheduled City and Police Department Orientation Classes or functions for new employees.
Pasadena police Department Regional Crime Laboratory
Training Guide
Toxicology

Name__________________________________

The employee will date and initial the following:

I have read the Laboratory Safety Manual ___________________________
I have read the Laboratory Operations Guide ___________________________
I have read the Toxicology Section Operational Guide _______________________
I have read the recommended reading materials ___________________________
I have watched the recommended safety videos ___________________________

Training Checklist

<table>
<thead>
<tr>
<th>Subject</th>
<th>Trainer’s Initials</th>
<th>Date Training Began</th>
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<tbody>
<tr>
<td>Controlled Substances</td>
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<td>Blood Alcohol Analysis</td>
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<tr>
<td>Urine Drug Analysis</td>
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<tr>
<td>Blood Drug Analysis</td>
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<td>Competency Testing</td>
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<tr>
<td>Written Examination</td>
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</tbody>
</table>
Certification of the completion of training

__________________________________________________________________________ Date__________________
Trainer's Signature

__________________________________________________________________________ Date__________________
Quality Manager Signature

__________________________________________________________________________ Date__________________
Laboratory Director Signature
GENERAL GUIDELINES

PURPOSE

The General Guidelines provide an overview of the function of the Toxicology section from analysis of body fluids, supervision of the breath alcohol testing program and court testimony. The training schedule outlines the required reading and practical applications for a six-month program.

TRAINING PROGRAM

All trainees receive intensive training lasting for approximately six months. The training includes the following areas:

- Laboratory Operations Guide
- Laboratory Safety Manual
- Security and handling of evidence
- Operation and maintenance of instrumentation and equipment
- All necessary paperwork
- Analysis of Evidence
- Court Testimony
- Breath Alcohol Technical Supervisor training

TOXICOLOGICAL EVIDENCE

Toxicological analysis will be performed on various types of evidence received in the laboratory. Examples include but are not limited to the following materials:

- Urine
- Blood
- Stomach contents
- Tablets and capsules
- Powders
- Liquids
- Miscellaneous (Food items, etc.)

TYPES OF EXAMINATIONS

Analysis of toxicological evidence received in the laboratory will use any or all of the following analytical techniques:
a. Screening Tests
   i. Toxi-Lab Chromatography
   ii. Immunoassay
   iii. Thin Layer Chromatography
   iv. Ultraviolet Spectroscopy
   v. Chemical Spot Tests

b. Confirmatory Tests
   i. Gas Chromatography Mass Spectrometry (GCMS)
   ii. Gas Chromatography (for ethanol quantitation)
   iii. Fourier Transform Infrared Spectroscopy (FTIR)

PROFICIENCY TESTING

Toxicology specimens will be commercially purchased to determine the proficiency of the analyst in toxicological analysis. Blood specimens are received for blood alcohol proficiency testing and urine specimens are received for urine drug proficiency testing.

Additional proficiencies may be prepared internally as needed.
TOXICOLOGY OBJECTIVES

Perform analysis of blood and urine specimens with a short turnaround time.

- All priority requests from an Assistant District Attorney or officer should be analyzed before the agreed deadline.
- Prioritize blood alcohol and other alcohol before other cases.
- Analysis schedule will depend on caseload in the section and available personnel.
  - Blood alcohols should be analyzed within one (1) week of receipt.
  - Blood drugs should be analyzed within one month.
  - Urine drugs should be analyzed within one month.
  - All other evidence should be worked within 2 months.
- Submit all evidence within one week after analysis is complete.
- File reports within one week of completion of analysis.

Provide training for the department and other agencies as needed.