Idaho State Police
Forensic Services
Toxicology Section

Section One

1.0 Screening of Blood and Urine for Drugs of Abuse by Enzyme Immunoassay

1.1 BACKGROUND

ELISA is an acronym for enzyme-linked immunosorbent assay. An ELISA is an enzyme immunoassay (EIA) in which one reactant is immobilized on a solid phase and the signal generator is an enzyme. The enzyme delivers a signal to indicate to what extent a particular antigen-antibody reaction has occurred. This reaction takes place inside of a polystyrene microtiter plate well. An enzyme commonly employed as a signal generator is horseradish peroxidase (HRP). The small size of HRP, the ease with which small conjugates can be produced through oxidation of its carbohydrate moieties to reactive aldehyde, its rapid kinetics, and reasonable price, make it popular in ELISA.

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Each OraSure Micro-Plate EIA is a competitive micro-plate immunoassay for the qualitative determination of a specific drug, or class of drugs in blood and urine. Each of the serum and oral fluid assays requires a predilution step for samples, controls and calibrators. This brings the analytes into an acceptable range for optimum performance of the bound microplate antibodies. Dilutions are either performed manually with an air displacement pipette or utilizing a Hamilton Dilutor. Samples, calibrators or controls are added to individual wells of the microplate along with the conjugate, which is the drug or hapten labeled with the enzyme horseradish peroxidase (HRP). There is a competition between the free drug in the matrix sample (blood or urine) and drug bound to enzyme (conjugate) for antibody (sheep or rabbit) fixed on the well. The wells are washed with DI water, the substrate (3,3′,5,5′-tetramethylbenzidine (TMB) with peroxide (H_2O_2) is added, and a color is produced. HRP catalyzes H_2O_2 oxidation of the substrate by transferring one electron from the TMB to the peroxide to yield a blue colored product. The reaction is stopped when 2.0N sulfuric acid is added to the well. This acidic environment provides the necessary conditions for the loss of one more electron to produce the final yellow color. The acidic environment also serves to inactivate the enzymatic activity of the HRP. The resulting absorbance at 450nm is inversely proportional to the amount of drug present in the sample or standard. Consequently, a more intense yellow color results in a greater absorbance and indicates a lower concentration of drug in the sample. The OraSure Micro-Plate EIA kit utilizes two matrix matched calibrators, one containing no drug (negative calibrator) and one at the concentration corresponding to the accepted cut-off for the drug (cut-off calibrator). In addition, negative and positive controls are used to assess the performance of the kit.

The PersonalLAB™ (P-LAB) is an automated microplate analyzer for processing immunoenzymatic techniques developed on 96-well microplates. The analyzer automatically dispenses samples and all reagents required for an ELISA testing

procedure. In addition, the analyzer allows for the programming of incubation times and wash steps.

A **PROTOCOL** is a set of instructions that direct the PersonalLAB™ how to run a particular assay. Protocols exist for each of the assays utilized. Protocols define the volume required of standards, controls, and reagents, each assay's steps, instrument wash and incubation parameters, sample tip specifications and the validation criteria and reading parameters. The **PROFILE** is information the software uses to actually process the samples and generate results. A **PROFILE** is a set of instructions, which direct the PersonalLAB,™ how to run a particular group of assays. The **PROFILE** includes the order of performance, the plate location(s), the standard/reagent location and the processing mode (parallel or serial dispensing).

1.2 SCOPE

OraSure Micro-plate assay kits intended use is for the qualitative screening for drugs-of-abuse in biological samples. The kits are either serum (S) or oral fluid (OF) based with appropriate dilutions made for application to the screening of blood and urine. The outcome of the assay is intended as only a preliminary analytical test result. The presence of a particular drug compound must be verified through analysis with a confirmatory instrument such as a gas chromatograph equipped with a mass selective detector.

As indicated in the table below, each assay in use has an established administrative threshold or cut-off. For this reason, a negative result does not indicate that no drug was present, only that the concentration was less than the administrative cut-off. For this reason there may be situations where confirmation of an analyte may be pursued even if the P-LAB indicates a negative result for the compound or a class of compounds in question. The exceptions are discussed in section 1.8.2.

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Assay	*Calibrator*	*Urine Cut-off*	*Blood Cut-off*
Amphetamine Specific Serum	d-Amphetamine	1000ng/mL	50ng/mL
Barbiturate Serum	Secobarbital	200ng/mL	100ng/mL
Benzodiazepine Oral Fluid	Oxazepam	300ng/mL	100ng/mL
Cannabinoid Oral Fluid	11-Nor-9-Carboxy-THC	50ng/mL	15ng/mL
Cocaine Metabolite Oral Fluid	Benzoylecgonine	300ng/mL	50ng/mL
Methadone Oral Fluid	Methadone	300ng/mL	50ng/mL
Methamphetamine Serum	Methamphetamine	1000ng/mL	50ng/mL
Opiate Serum	Morphine	300ng/mL	50ng/mL

1.3 EQUIPMENT

1.3.1 <u>Sample Dispensing Options</u>

1.3.1.1 Air-displacement pipettes and appropriate tips.

1.3.1.2 Repeater Pipette and appropriate tips.
1.3.1.3 Hamilton MicroLab® 500A series dilutor or equivalent, equipped with appropriate syringes.

1.3.2 Plasticware
1.3.2.1 5mL disposable plastic sample tubes (Adaltis 55484 or equivalent)
1.3.2.2 75mL plastic reservoirs (Adaltis MV3007 or equivalent)
1.3.2.3 35mL plastic reservoirs (Adaltis MV3006 or equivalent)
1.3.2.4 5mL plastic sample cups (Adaltis PF2026 or equivalent)
1.3.2.5 Caps for cups (Adaltis LT65649 or equivalent)
1.3.2.6 Disposable plastic pipette tips (Adaltis LP1181, no substitutions)

1.3.3 PersonalLAB™ automated microplate analyzer

1.3.4 Tube Rocker (Fisher Scientific or equivalent)

1.4 REAGENTS

1.4.1 OraSure Technologies Assay Kits

- The OraSure kits contain the following items:
- Micro-plates coated with anti-drug antibodies.
- Enzyme conjugate for specific drug/drug class.
- TMB substrate reagent (universal).
- 2N H_2SO_4 Stopping reagent (universal).
- Laboratory Specification Sheet
- Package Insert

1.4.2 Processing of New Assay Supplies

1.4.2.1 When a new kit is opened note the expiration date of all components listed on each assay's specification sheet.

1.4.2.1.1 If a lot number has changed, update lot number information on coversheet for original data printout.

1.4.2.1.2 Date and initial kit specification sheet and indicate whether it is a five or a two-plate kit.

1.4.2.1.3 Check the revision date for the package insert. If the revision is not in the OraSure/PersonalLAB binder, place it in the appropriate section.

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1.4.2.2 When new urine calibrators and controls arrive note expiration date and if necessary, update lot number information on coversheet for original data printout.

1.4.2.3 **Cocaine Assay Conjugate Preparation**

1.4.2.3.1 Using a calibrated pipette, to the vial containing *Benzoylecgonine Lypophilized Stock Enzyme Conjugate*, add 2mL *Conjugate Diluent.*

1.4.2.3.2 Place vial on tube rocker for a minimum of 10 minutes.

1.4.2.3.3 Using a calibrated pipette add the volume of reconstituted *Stock Enzyme Conjugate* listed on the kit package insert to 10mL of *Conjugate Diluent.* The volume of *Stock Enzyme Conjugate* is lot specific. *Prepare only necessary volume of conjugate.*

1.4.2.3.4 Gently mix Conjugate Diluent bottle on tube rocker for a minimum of 1 minute.

1.4.2.3.5 Prior to use, allow bottle to equilibrate for a minimum of 30 minutes at room temperature or overnight under refrigeration.

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1.5 REFERENCE MATERIAL

For both urine and blood the following calibrators and controls must be included in each analysis run.

1.5.1 Urine

1.5.1.1 **Calibrators**

1.5.1.1.1 Negative Urine Calibrator
OraSure 60421 or equivalent.

1.5.1.1.3 Cut-off Urine Calibrator
OraSure 61423 or equivalent.

1.5.1.2 **Reagent and Standards/Controls Rack Controls**

1.5.1.2.1 Negative Urine Control
OraSure 61422 or equivalent.
This should be used for all assays except benzoylecgonine. Refer to 1.5.1.2.2 for benzoylecgonine negative control preparation.

1.5.1.2.2 Benzoylecgonine Negative Control
Dilute 500µL Cut-off Urine Calibrator with 500µL Negative Urine Calibrator. Mix well.

1.5.1.2.3 Positive Urine Control
OraSure 61424 or equivalent.

1.5.1.3 **Sample Rack Controls**

1.5.1.3.1 Negative control urine
In house, Utak or comparable vendor.

1.5.1.3.2 Positive drugs-of-abuse control urine
BioRad, UTAK or comparable vendor.

1.5.2 Blood

1.5.2.1 **Stock Standard Solutions**
Drug standards should be obtained from Cerilliant, Alltech, Sigma or equivalent vendor. Different vendors should be used to make up the *Calibrator* and *Control* Working Solutions. Certificates of analysis should be stored centrally.

Stock (1.0mg/mL)	Potential Vendor
S-(+)-Amphetamine	Cerilliant A-008 1.0mg/mL
S-(+)Methamphetamine	Cerilliant M-020 1.0mg/mL
Benzoylecgonine	Cerilliant B-004 1.0mg/mL
Methadone	Cerilliant M-007 1.0mg/mL
Morphine	Cerilliant M-005 1.0mg/mL
(-)-11-nor-9-Carboxy-Δ9-THC	Cerilliant T-018 100µg/mL
Oxazepam	Cerilliant O-902 1.0mg/mL
Secobarbital	Cerilliant S-002 1.0mg/mL

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1.5.2.2 **Working Standard Solution**
Fill 10mL volumetric flask ~½ full with methanol. Add 50µL each of stock amphetamine, methamphetamine, benzoylecgonine, methadone and morphine. Add 100µL oxazepam and secobarbital*. Add 150µL c-THC. QS to 10mL with methanol. Record lot numbers of stock standards on reagent log.

Solution is stable for 12 months when stored at 4°C.

*Include secobarbital when barbiturate screen is used.

1.5.2.3 **Blood Calibrators**

1.5.2.3.1 Negative Whole Blood Calibrator
Utak 44600-WB (F) or equivalent.

1.5.2.3.2 Cut-off Calibrator

1.5.2.3.2.1 **Direct Spiking Preparation**
To 1mL of negative blood (Utak 44600-WB (F) or equivalent) add 10µL working standard solution.

1.5.2.3.2.2 **Serial Dilution Preparation**
Prepare 300% of cut-off solution as described below in 1.5.2.4.1. To 1mL of negative blood (Utak 44600-WB (F) or equivalent) add 500µL 300% blood stock.

1.5.2.4 **Reagent and Standards/Controls Rack Controls**

1.5.2.4.1 Direct Spiking Preparation
To 1mL of negative blood (Utak 44600-WB (F) or equivalent) add working standard solution as indicated below.

Control Type	% of cutoff	Working Standard Solution
Negative	50%	5µL
Positive	300%	30µL

1.5.2.4.2 Serial Dilution Preparation
Prepare 300% of cut-off solution as described in 1.5.2.4.1. Refer to table below for additional dilutions.

Control Type	% of cutoff	Whole Blood Stock	Whole Blood Dilution
Negative	50	500µL of 100%	500µL
Positive	100	500µL of 300%	1000µL

1.5.2.5 **Sample Rack Blood Controls**

1.5.2.5.1 Negative Whole Blood
Utak 44600-WB (F) or equivalent.

1.5.2.5.2 Positive Whole Blood
Positive controls may be prepared and/or commercially obtained. The drug concentration may be varied.

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1.5.2.5.2.1 **125% Positive Whole Blood**
Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with quality control working standard solution at 125% of cutoff. To 2mL of negative blood add 25μL quality control working standard solution.

1.6 PROCEDURE

1.6.1 General Rules of Operation for the PersonalLab

1.6.1.1 Care should be taken to not impede the arm action.

1.6.1.2 Run instrument with the top down. Having the top down is safer for the operator and better for the substrate.

1.6.2 Initial Processing of Samples

1.6.2.1 On the Toxicology Submittal Form record the condition of the inner kit seals.

1.6.2.2 On toxicology analysis worksheet record the following information:

- Description/type of sample collection kit
- Condition of inner seal
- Type and number of specimen container(s)
- Condition of specimen container seals.
- Kit lot number and expiration date.

1.6.2.3 Place laboratory number on each sample container.

1.6.2.4 When two samples are present, the samples should be labeled "A" and "B" or equivalent.

1.6.2.5 If particulates or clots are visible in a blood sample, homogenize with tissue grinder or clarify by centrifuging.

1.6.2.6 Urine samples with an unusually high turbidity can be centrifuged prior to analysis.

1.6.2.7 Urine samples preferably should not contain the preservative sodium azide.

1.6.3 Sample Dilution

1.6.3.1 **Dilution Dispensing Options**

1.6.3.1.1 Option one:

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Calibrated air-displacement pipettes and appropriate tips.

1.6.3.1.2 Option two:
Calibrated Repeater Pipette and appropriate tips.

1.6.3.1.3 Option three:
Hamilton MicroLab® 500A series dilutor equipped with appropriate calibrated sample and reagent/diluent syringes.

1.6.3.2 **Dilution Volumes**

1.6.3.2.1 1 in 5 parts dilution

Sample	Forensic Diluent
160µL	640µL
200µL	800µL
250µL	1000µL

1.6.3.2.2 1 in 60 part dilution

Sample	Forensic Diluent
91µL / 1:5 dilution	1000µL
15µL	885µL

1.6.3.3 **Appropriate Dilution for Each Assay**

1.6.3.3.1 Urine

Dilution	Assays *Grouped for PROFILE*
1 in 60	Benzodiazepines (OF), Cocaine Metabolite, and Methadone
1 in 60	Amphetamine, Methamphetamine, Cannabinoids and Opiates
1 in 5	Barbiturates

1.6.3.3.2 Blood

Dilution	Assays *Grouped for PROFILE*
1 in 5	Barbiturates
1 in 5	Amphetamine,

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	Benzodiazepines, Methadone, and Methamphetamine
1 in 5	Cocaine, Opiates, and Cannabinoids

1.6.4 Preliminary Tasks

1.6.4.1 Fill wash bottles with distilled water.

1.6.4.2 Check pipette tip tray supply. If necessary, fill with Adaltis disposable tips. If tips are replaced, reset tip counter (1.6.7.11).

1.6.4.3 Check printer paper supply. Refill if necessary.

1.6.4.4 Remove samples and reagents from refrigerator ≅ one hour prior to starting analysis.

1.6.4.5 Prepare samples for analysis. Dilute as indicated under section 1.6.3.3.

1.6.5 Session Preparation

1.6.5.1 Turn on computer.

1.6.5.2 Double click on *PersonalLAB 2.2a-SP3* icon.

1.6.5.3 Click on Folder 🗀 icon. Prompt for User Name and Password will come up. Password is case sensitive.

1.6.5.4 Open screen comes up. Select ⊙ *Session* and Click **OK**

1.6.5.5 From Open Session Screen, select appropriate template from list.

1.6.5.6 Template will show up in lower "file list" box. Note file name for run for further reference. **Double click** in box on selection or click **OK**

1.6.5.7 *Session* screen will now come up. Click on test tube icon (third from right) to bring up *Session - Sample Programming* Screen.

1.6.5.8 To clear previous programming for first analysis run of the day, click on Clear Sample Rack.

1.6.5.9 On *Session - Sample Programming* screen input in the "ID range" box the laboratory number of specimen or the source

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of information for positive and negative controls. The "ID range" box is located on the left portion of the screen. Information entered will appear on the right portion of the screen after Enter or ADD ID is pressed. Use only numbers, letters, or dashes with no spaces.

1.6.5.10 To select all assay PROTOCOLS in the PROFILE, double click left on rack icon. Screen will turn blue. By clicking on the rack icon, "X"s are placed on all assays indicating that the sample will be analyzed by all indicated PROFILES.

1.6.5.11 To choose selected assay PROTOCOL(S), highlight desired sample boxes under assay and double click right mouse button while cursor is in highlighted area.

1.6.5.12 If running an additional batch of assays or if rerunning samples DO NOT PRESS CLEAR SAMPLE RACK or laboratory numbers and control information will have to be reentered. For **second** run, click twice on rack symbol which is located to the left of the assays included in the PROFILE. This will remove the {} around the programmed information (laboratory numbers, control information).

1.6.5.13 Click DONE

1.6.5.14 *Session – Protocol Position"* screen should now be displayed. This view of the plate racks illustrates the number and position of the individual strips, which are necessary for each individual assay.

1.6.5.15 **Load plate racks with appropriate strips.**
Press down on the strips to insure they are seated firmly into the tray. Improper strip positioning can result in the strip popping up and the instrument jamming during the washing or incubation step.

1.6.5.16 Click DONE

1.6.5.17 Save *Session* by clicking on save or on 🖫 (save session). ***This step is crucial to prevent software glitch. Do not attempt to proceed to next step without first saving session.***

1.6.5.18 Turn on instrument.

1.6.5.19 Load sample rack.

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1.6.5.20 From *Session* Screen, click on *Start Session* icon (far right/red arrow).

1.6.5.21 *Profile –Vial Locations* view appears. Load cups and reagent reservoirs onto platform.

1.6.5.21.1 Using the screen template, place the 35mL conjugate containers, and the 75mL substrate and stop reservoirs according to their designated location on the platform.

1.6.5.21.2 Place appropriately diluted amount of controls (negative and positive) and calibrators (negative and cutoff) into the 5mL cups. Refer to section 1.6.3.3 for appropriate dilutions. Place the cup at its designated numbered location.

1.6.5.22 Note dead volume for each container listed below.

Plasticware	Dead Volume
5mL disposable plastic culture tubes	200µL
75mL plastic reservoirs	1.5mL
35mL plastic reservoirs	1.0mL
5mL plastic cups	200µL

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1.6.5.23 After loading is complete, hit Continue.

1.6.5.24 Start-up screen comes up. Screen will indicate *Waiting for Instrument Initialization.*

1.6.5.25 If the lid is open, the screen will indicate *"Warning! Interlock disabled. Continue?* Respond YES for the instrument to continue operation.

1.6.6 Pre-run Maintenance

1.6.6.1 From *Session Status Box* screen, on Session Browser tab, click on OPERATION MONITOR tab.

1.6.6.2 Access *Maintenance* on OPERATION MONITOR from START UP pull down or click on station maintenance icon (second from left).

1.6.6.3 From MAINTENANCE screen, click on SELF TEST tab. To start self test click on START

1.6.6.4 When the *Self Test* is complete, the program will inquire *Print Self-Test Report?* Click Yes

1.6.6.4.1 *Self-Test Report* should be placed in P-LAB maintenance binder or stored with original data.

1.6.6.4.2 A copy of the *Self-Test Report* may be placed in the casefile.

1.6.6.5 If *Self Test* indicates that the instrument has passed all evaluations, proceed with daily maintenance. Click on tab for DAILY MAINTENANCE.

1.6.6.6 Click on Fill Syringe after priming, screen will inquire *Continue?* Indicate Yes if bubbles are observed. Press No when bubbles are no longer present.

1.6.6.7 Click on Fill Lung Screen will instruct operator to open front cover to view lung filling. Click **OK**. After an initial fill, screen will inquire re: *250 µl more.* If lung is not between = lines on lung, press Yes. Screen will continue to inquire until operator observes that lung is sufficiently full and selects No. Screen will now instruct operator to *close cover to continue operation*. Click OK.

1.6.6.8 Click on Buffer (TANK) 1 Prime.
Watch tubing lines for bubbles. Continue priming until no bubbles are present.

1.6.6.9 Click on Buffer (TANK) 2 Prime.
Watch tubing lines for bubbles. Continue priming until no bubbles are present.

Note: Buffer Ext Prime is not used.

1.6.6.10 A ✓ mark will appear when each task is complete.

1.6.6.11 To reset tip counter, access *Reset tips* from START UP pull down or click on Reset Tips icon (3rd from left).

1.6.7 Run Execution

1.6.7.1 From *Operation Monitor* folder tab, click on EXE ▸ icon (4th from left).

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1.6.7.2 Instrument will remind you about rack placement. Operator can *retry* if sample rack is not in place.

1.6.7.3 Screen will indicate *Waiting for Lamp Warm-up.* Instrument will check strip/racks in carriages.

1.6.7.4 To monitor run, click on profile tab.

1.6.7.5 When run is complete *Processor Screen* will indicate *Session Terminated. Print the Operation Monitor log to troubleshoot any problem: it will not be possible to print it after you have closed the Processor.* If End (of) Work is not to be pursued at this point, click on **OK** followed by ☒. Screen will inquire *Exit from WB Processor?* Click on Yes.

1.6.7.6 To perform an additional run after results are printed, select ⊙ *Session* (1.6.6.4) from *Open Screen* and proceed as before.

1.6.8 Obtaining Results - Post-Run

1.6.8.1 From *WorkBench* screen, click on Open Results icon (third from right) or Open icon and select ⊙ *Results.*

1.6.8.2 Highlight session name of choice under *File List.*

1.6.8.3 Click OK

1.6.8.4 Select Report icon (far right). "*FETCHING*" (data processing) will commence.

1.6.8.5 When processing of data is complete, the Report Browser screen will appear. To print results click on Assay/Profile tab for each assay or select assay from profile list on Report Index tab. The box to the right of the Print icon should have *Light Reporting* selected.

1.6.8.6 After data has printed, click ☒, click ☒ to close out screens.

1.6.9 Obtaining Archived Results

1.6.9.1 Click on Open Folder icon and select ⊙ Results.

1.6.9.2 On Database Results tab, Highlight Session ID, Name or Entry Date.

1.6.9.3 Click OK. *Results* page comes up.

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1.6.9.4 Click on report icon. After data processing is complete *Report Browser* will appear. To print results click on Assay/Profile TAB for each assay or select assay from profile list on Report Index tab. The box to the right of the Print icon should have *Light Reporting* selected.

1.6.9.5 After data has printed, click ☒, click ☒ to close out screens.

1.6.10 Post-run Tasks

1.6.10.1 **General Clean-up**

1.6.10.1.1 Return conjugates, stop and diluent reservoirs to refrigerator.

1.6.10.1.2 Dispose of used calibrator, controls, micro-plates, used tips in drawer and samples into appropriate biohazard container.

1.6.10.2 **Instrument Shut-down**

1.6.10.2.1 For *End-of-Work* routine, access *Maintenance* on OPERATION MONITOR from START UP pull down or click on station maintenance icon (second from left).

1.6.10.2.2 Click on End Work

1.6.10.2.3 Display will instruct operator *Please fill in buffer 2 with distilled water*. Click OK

1.6.10.2.4 Screen will direct operator to *Please, empty waste tank*. Click OK after depressing button on left side of instrument. This is a gravity flow water system and the button must be held in for it to fully empty.

1.6.10.2.5 Screen will direct operator to *Please, empty used tips drawer*. Click OK

1.6.10.2.6 Screen will advise operator *Do not forget to turn the instrument off after you have closed the Processor.*

1.6.10.2.7 When End Work is complete click OK

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1.6.10.2.8 Click on ☒ to close *WorkBench* window. Screen will inquire *Exit from WB Processor?* Select [Yes].

1.6.10.2.9 Exit from program and use software to shut down computer.

1.6.10.2.10 Turn off instrument.

1.7 RUN ACCEPTANCE CRITERIA

1.7.1 P-LAB Calibrators and Controls

1.7.1.1 The individual replicates for the absorbance of the *negative calibrator* must be less than 1.2 times the mean *negative calibrator* and greater than 0.8 times the mean *negative calibrator*.

1.7.1.2 The individual replicates of the *cut-off calibrator* must be less than 1.2 times the mean *cut-off calibrator* and greater than 0.8 times the mean *cut-off calibrator*.

1.7.1.3 The mean absorbance for the *negative calibrator* is greater than the absorbance for the *negative control*.

1.7.1.4 The absorbance for the *negative control* is greater than the mean absorbance for the *cut-off calibrator*.

1.7.1.5 The mean absorbance for the *cut-off calibrator* is greater than the absorbance for the *positive control*.

1.7.2 Urine and Blood Sample Controls

1.7.2.1 Matrix matched urine and blood controls, analyzed as samples, should indicate an appropriate positive or negative response.

1.7.2.2 For purposes of this criterion, a significantly depressed absorbance qualifies as a positive result.

1.8 INTERPRETATION OF RESULTS

1.8.1 Positive Result

A positive result for a sample is indicated by an absorbance less than or equal to the *Cut-off Calibrator*.

1.8.2 Depressed absorbances

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At the discretion of an analyst, confirmatory techniques may be applied to samples that exhibit depressed absorbances. For purposes of this exception, depressed absorbances are those which fall between the value observed for the cut-off calibrator and the negative control. If data for confirmatory techniques supports the presence of an analyte, the analyte may be reported as present. This is especially applicable if the cross-reactivity for the analyte of interest is known to be low. Examples of cases where this exception could apply include infant testing and samples collected as the result of a drug recognition examination (DRE).

1.8.3 Negative Result

A negative result for a sample is indicated by an absorbance that is greater than the Cut-off Calibrator. Special considerations may apply as outlined in above (1.8.2).

1.9 DISTRIBUTION OF ASSAY INFORMATION

1.9.1 Assay results are to be recorded on the case file toxicology analysis form.

1.9.2 A cover sheet containing the date of the run and the lot number for each assay should be included with original data. This original data will be stored centrally in the laboratory where the analysis was performed in the location designated for the storage of the assay printouts until archiving.

1.9.3 A copy of assay results need not be included in individual case files. When necessary, a copy of the control and standard printouts may be prepared from the centrally stored document.

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1.10 P-LAB MAINTENANCE

1.10.1 When-in-use Daily End-of-day Maintenance/Tasks

1.10.1 Inspect for fluid in the vacuum pump condensation trap.

1.10.2 Wipe down instrument with 70% isopropanol.

1.10.2 Periodic Maintenance

The extent of P-LAB use should be used as the indicator of when periodic maintenance is warranted. The following is intended as suggested maintenance frequency. The schedule should be adjusted according to individual laboratory needs. However, the tasks should be performed a minimum of every 3 months.

1.10.2.1 **Monthly Maintenance**

1.10.2.1.1 Wash, air dry and replace the Wash Tanks water.

1.10.2.1.2 Wipe the outside off the removable sample needle with 70% isopropanol.

1.10.2.1.3 Disinfect the discard tip tray by rinsing with 70% isopropanol.

1.10.2.2 **Quarterly Maintenance**

1.10.2.2.1 Wash Needle Station Disinfecting
Add 70% isopropanol to needle wash station.
Brush with small brush. Remove isopropanol.
Rinse well with DI water.

1.10.2.2.2 Wash Tank (black and red top)/Line Disinfecting
Place 10-15% isopropanol in wash tanks
Circulate alcohol 3-5 times.
Wipe off floats.
Replace isopropanol with fresh DI water.
Circulate water 5-7 primes to rinse system.

1.10.2.2.3 Needle cleaning tank (white top)
Place 14% isopropanol/MeOH in tank.
Allow to soak for 10-15 minutes.
DO NOT PRIME.

1.10.2.2.4 Wash Head Stylet Cleaning
Turn off instrument.
Run stylets through wash head tips.
Clean wash head tips with 70% isopropanol.
Move arm back to home (far left).

1.10.2.2.5 Removable/sample needle cleaning
Soak needle in 10-15% isopropanol.

1.10.3 **Preventative Observations**

1.10.3.1 Watch wash head process plates to verify that head is functioning properly.

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1.11.4 OraSure Technologies Package Inserts for Serum Microplate EIA.

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Idaho State Police
Forensic Services
Toxicology Section

Section One
Screening of Blood and Urine for Drugs of Abuse

Procedure: Background and Standard Operating Procedure for Screening of Whole Blood and Urine by Enzyme Immunoassay

Revision #	Issue Date	History
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1	04-24-02	Updated and made STC name change corrections
2	09-13-02	Clarification of distribution of assay information (1.7.1)
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Approval

Technical Leader: ______________________ **Date:** __________
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Issuance

QC Manager: ______________________ **Date:** __________
Richard D. Groff

2.2.1: Toxi-Lab Toxi-A Drug Dectection System (2.1.1.1 Toxi Lab Toxi-A Instruction Manual)

Orig rev. 0

TOXI-LAB® A DRUG DETECTION SYSTEM

INSTRUCTION MANUAL

Cat. No. 181A

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TOXI-LAB, Inc.
Irvine, CA 92718

TOXI-LAB® A DRUG DETECTION SYSTEM INSTRUCTION MANUAL

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1.0 INTRODUCTION

1.1 Description and Intended Use

TOXI-LAB® A Drug Detection System is a rapid thin-layer chromatographic method for the separation, detection and identification of analgesics, stimulants, tranquilizers, and antidepressants (basic and neutral drugs). TOXI-LAB A may be used by hospital, outpatient, reference, and forensic laboratories to screen for the presence of drugs in biological fluids and other samples in solid or liquid form.

For forensic testing purposes (i.e., drugs of abuse testing), the TOXI-LAB A Drug Detection System provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgement should be applied to any test result, particularly when preliminary positive results are used.

The TOXI-LAB AB Initial System includes materials required for TOXI-LAB A and TOXI-LAB B. This manual discusses the materials and procedures required for TOXI-LAB A only. For information on TOXI-LAB B, refer to the TOXI-LAB B Drug Detection System Instruction Manual.

1.2 Principle

TOXI-LAB A is a modification of the thin-layer chromatography (TLC) technique; the procedural steps of extraction, concentration, inoculation, development, and detection have been simplified for convenience, and to minimize sample processing time.

Drugs are extracted from biological fluids and other materials using TOXI-TUBES® A containing a mixture of solvents and buffering salts that extract basic and neutral drugs. The solvent extracts are concentrated by heat and evaporation, depositing the unknown drugs onto discs of chromatographic media. The dried discs are then inserted (inoculated) into the center openings of TOXI-GRAMS® A.

The "loaded" chromatograms are developed by placing them in developing chambers containing small volumes of organic solvent. Elution of the unknown and standard drugs from the discs and the resulting position (R_f) of these drugs on the chromatograms occur during migration of the developing solvent. Detection of the unknown and standard drug spots is achieved when the chromatograms are dipped into chromogenic reagents. Identification is based on matching a drug spot in the unknown zone with an adjacent standard drug spot having the same R_f, size, shape, and color characteristics.

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2.0 REAGENTS AND MATERIALS

2.1 Materials Supplied in TOXI-LAB A

2.1.1 TOXI-KIT A-50 (Cat. No. 101A-50)

2.1.1.1 TOXI-GRAMS A-50 (Cat. No. 106A-50)
Drug-standardized 6-channel chromatograms for separation of basic and neutral drugs; made of glass microfiber paper and impregnated with silicic acid and vanadium salt. TOXI-DISCS® A-1, A-2, A-3, and A-4, impregnated with a total of 26 drugs and a dye marker, are pre-inserted into four of the six openings. See Appendix I for drug standards.

2.1.1.2 TOXI-TUBES A-50 (Cat. No 109A-50)
Tubes for extraction of basic and neutral drugs; contain a mixture of solvents, buffering salts, and a phase-marking dye.

2.1.1.3 TOXI-DISCS® Blank A-100 (Cat. No. 108A-100)
Discs for concentration of drugs; made of glass microfiber paper and silicic acid.

2.1.1.4 Chemicals for Preparation of TOXI-DIP® A-3 Reagent (Cat. No. 111A)
Solution for making TOXI-DIP A-3 Reagent; contains potassium iodide, iodine, and bismuth subnitrate; 7.5 mL of methanolic solution in one vial.

2.1.1.5 TOXI-LAB A Worksheets (Cat. No. 187A)
Worksheets showing TOXI-GRAMS A detection stages and standards; for recording observations. 50 sheets/pad.

2.1.2 TOXI-GRAMS Blank A-50 (Cat. No. 120A-50)
Nonstandardized 6-channel chromatograms for separation of basic and neutral drugs; made of glass microfiber paper and impregnated with silicic acid and vanadium salt. (For use with TOXI-DISCS drug standards.)

2.1.3 TOXI-CONTROL® Six Pack (Cat. No. 170G)*
Package of 6 urine controls (1 negative, 5 positives); contains 1 vial each of TOXI-CONTROL Nos. 1, 2, 3, 4, 5, and 6; 50 mL/vial.

2.1.4 TOXI-CONTROL No. 19 (Cat. No. 191AB)*
Positive urine control; 50 mL/vial.

2.1.5 TOXI-DISCS LIBRARY II Set (Cat. No. 131N)
Set of 12 vials of TOXI-DISCS containing a total of 41 drug standards.

2.1.6 Ethyl Acetate TOXI-LAB Grade (Cat. No. 202)*
Pretested ethyl acetate for use with TOXI-LAB.

2.2 Equipment and Accessories Supplied in TOXI-LAB A

2.2.1 TOXI-LAB Workstation (Cat. No. 150)*

2.2.1.1 Workstation Module I (Cat. No. 151)
Work center for sample preparation; includes Storage Tray, Heat Gun, and Heat Gun Holder.

2.2.1.2 Workstation Module II (Cat. No. 154)
Work center for drug detection; includes turntable, dipping jars with special caps and stand-off jar. (Also includes extra storage jar cap and friction band.)

*Items common to TOXI-LAB A and TOXI-LAB B.

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2.2.1.3 Workstation Module III (Cat. No. 155)
Work center for viewing TOXI-GRAMS with ultraviolet light.

2.2.1.4 OMEGA-12 Extraction Solvent Concentrator with Screen (Cat. No. 153)
Solvent concentrator with 12 wells for Disposable Concentration Cups, and screen cover.

2.2.1.5 Disposable Concentration Cups (Cat. No. 152)
Disposable aluminum cups for evaporation of extraction solvents.

2.2.2 Electric Warmer with Protective Sheet (Cat. No. 118)*
Low-heat source for solvent evaporation.

2.2.3 Ultraviolet Light (Cat. No. 116)*
UVL-21 Blak-Ray, long-wave UV light (365 nm) for viewing TOXI-GRAMS.

2.2.4 Microdispenser (Cat. No. 190L)*
Drummond Dialamatic Microdispenser; positive displacement pipet with variable volume to 50.0 µL.

2.2.5 TOXI-RACK® 3 Set (Cat. No. 113C)*
Multiple development racks for developing up to three TOXI-GRAMS simultaneously; includes Small Chromatography Jars with Cap.

2.2.6 Forceps (Cat. No. 184)*
Stainless steel forceps for handling TOXI-GRAMS.

2.2.7 Disc-handling Pins (Cat. No. 186)*
Pearl-headed pins for handling TOXI-DISCS.

2.2.8 Chromatogram Holder (Cat. No. 163)*
Clip for holding chromatograms while dipping.

2.2.9 Ammonium Hydroxide Vial with Cap*
Small amber vial for storing small volumes of ammonium hydroxide.

2.2.10 Small Chromatography Jar with Cap (Cat. No. 113A)*
Glass chamber for development of TOXI-GRAMS.

2.2.11 TOXI-DIP A-3 Stock Bottle (Cat. No. 119C)
Labeled, 500-mL capacity bottle for storing TOXI-DIP A-3 Reagent stock solution.

2.2.12 TOXI-LAB A Developing Solution Bottle (Cat. No. 112A)
Labeled, 100-mL capacity bottle for storing TOXI-LAB A Developing Solution.

2.3 Reference and Educational Aids Supplied in TOXI-LAB A

2.3.1 TOXI-LAB A Drug Detection System Instruction Manual (Cat. No. 181A)
Instruction manual for materials and procedures involved in the TOXI-LAB A Drug Detection System.

2.3.2 Color Detection Guide A (Cat. No. 182A)
Color photograph showing TOXI-GRAMS A drug standards in four detection stages.

2.3.3 TOXI-LAB A Drug Characteristics Table (Cat. No. 185A)
Listing of R_f values and color characteristics for over 70 drugs as seen on TOXI-LAB A.

*Items common to TOXI-LAB A and TOXI-LAB B.

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2.3.4 TOXI-LAB Drug Compendium (Cat. No. 201)*
Compendium of drug and pharmaceutical information, full-color PHOTOGRAMS® showing detection characteristics of drugs and metabolites, and TOXI-TIPS® Technical Information and Procedures.

2.3.5 TOXI-LAB AB Training Video Tape (Cat. No. 230)*
VHS video tape of the TOXI-LAB A Procedure; also includes demonstrations of the TOXI-LAB B Procedure, TOXI-LAB Validation Procedure, and fundamentals of troubleshooting.

2.4 Reagents Required for TOXI-LAB A

2.4.1 Ethyl acetate TOXI-LAB Grade (Cat. No. 202) or equivalent.*

2.4.2 Ammonium hydroxide, approximately 58% NH_4OH, or 28% to 30% NH_3, reagent grade.*

2.4.3 Sulfuric acid, concentrated, reagent grade.*

2.4.4 Methanol, reagent grade.

2.4.5 Glacial acetic acid, reagent grade.

2.4.6 formaldehyde, approximately 37% HCHO, reagent grade.

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*Items common to TOXI-LAB A and TOXI-LAB B.

3.0 PROPER HANDLING AND STORAGE OF MATERIALS AND SPECIMENS

3.1 Chemicals and Reagents
Read labels and adhere to cautions and warnings. Follow instructions for reagent preparation and storage, Section 4.0.

3.2 TOXI-GRAMS
TOXI-GRAMS are fragile and susceptible to contamination. Handle carefully with clean forceps.

Store standardized TOXI-GRAMS at 15°-30°C in the desiccant-containing jars provided. If the desiccating crystals turn pink, remove them and reactivate by heating at approximately 100°C until they turn blue, then return them to the jar. Adsorption of moisture may cause poor resolution and/or deterioration of the standard drugs. DO NOT USE if this is observed when chromatograms are tested (see Section 8.0, Quality Control Procedure). Desiccation is not necessary for nonstandardized (blank) chromatograms.

3.3 TOXI-DISCS
Use disc-handling pins (touching the discs lightly) and clean press cards when handling TOXI-DISCS. Strips of index card material, or similar paper material, may be used as press cards.

Store TOXI-DISCS drug standards at 15°-30°C in the desiccant-containing vials provided. If the desiccating crystals turn pink, remove them and reactivate by heating at approximately 100°C until they turn blue, then return them to the vial. Test the discs prior to use; adsorption of moisture may cause drug deterioration. DO NOT USE if this is observed when discs are tested.

3.4 Specimens
Avoid specimen contact with rubber objects or soft plastics; some rubber stoppers may produce artifacts with TOXI-LAB A (see "Artifact" in TOXI-TIPS Technical Information section of the TOXI-LAB Drug Compendium). Follow safe laboratory procedures and precautions for handling biological hazards.

Biological specimens should be refrigerated if the analysis is not to be performed immediately. See Section 7.0 for the preparation of non-urine specimens.

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4.0 REAGENT PREPARATION, PRECAUTIONS, AND STORAGE INSTRUCTIONS

4.1 TOXI-DIP A-1 Reagent (formaldehyde vapors)
Pipet approximately 25 mL of 37% formaldehyde solution through an opening in the stand-off to the bottom of the A-1 jar. Keep the top surface of the stand-off free of liquid; use a paper towel to wipe off any liquid residue on the stand-off. Date, and cap tightly. Strong formaldehyde fumes are essential; replace with fresh 37% formaldehyde solution weekly and dispose of properly. Use with adequate ventilation.

4.2 TOXI-DIP A-2 Reagent (sulfuric acid, concentrated)
Fill the A-2 jar with concentrated sulfuric acid to approximately 1/4 inch from the top of the jar (approximately 250 mL). Sulfuric acid may become contaminated with water vapors or formaldehyde; keep the A-2 reagent jar capped when not in use, and heat off the lower two-thirds of the chromatograms prior to dipping in TOXI-DIP A-2. Replace with fresh sulfuric acid if the reagent becomes contaminated, and dispose of properly.

CAUTION: This reagent is caustic. Handle with care. Use with adequate ventilation.

4.3 TOXI-DIP H_2O (water)
Fill the H_2O jar with deionized (or distilled) water to approximately 1/4 inch from the top of the jar (approximately 250 mL). Change the water daily or after every 5-10 chromatograms, and dispose of properly.

4.4 TOXI-DIP A-3 Reagent (modified Dragendorff's reagent)
Empty the contents of one A-3 chemical vial into the A-3 jar. Add 10 mL of glacial acetic acid. While stirring, dilute with deionized (or distilled) water to approximately 1/4 inch from the top of the jar (final volume approximately 250 mL). Reagent should appear opaque. Cap tightly and store at room temperature. As reagent is used, replenish from stock. Use with adequate ventilation. Dispose of properly.

4.5 Stock Developing Solution
To the developing solution bottle add 87 mL of ethyl acetate, 3 mL of methanol, and 1.5 mL of water. Cap tightly and mix well. Store at room temperature. (A larger volume may be prepared in a suitable container, and may be stored in a dispenser capable of delivering 3-mL aliquots.)

Formulate working solution for development, with ammonium hydroxide added, immediately before use (see Section 5.5.1). Avoid the use of plastic pipets, containers, and stoppers when preparing, storing and dispensing developing solution. Ethyl acetate may deteriorate with time. Use with adequate ventilation, and dispose of properly.

NOTE: Shelf life of detection reagents depends partly on frequency of use (number of chromatograms dipped into them). Detection of drug standards, as per Color Detection Guide A, indicates potent reagents. If poor detection is noted, replace reagents. See Quality Control Procedure, Section 8.0.

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5.0 TOXI-LAB A GENERAL PROCEDURE — URINE SPECIMENS

5.1 Preliminary Steps

5.1.1 Plug in the electric warmer.

5.1.2 Obtain the appropriate number of TOXI-TUBES A, TOXI-LAB A Worksheets, the Omega-12 Extraction Solvent Concentrator with Screen, and Disposable Concentration Cups. Label each with the respective sample numbers.

5.1.3 Allow specimens to reach room temperature before extraction.

5.2 Extraction

5.2.1 To TOXI-TUBES A, add the urine specimens up to the arrows indicating 5 mL. See Section 7.0 for the preparation of non-urine specimens.

5.2.2 Cap the tubes tightly and mix well by gentle inversion for a minimum of 2 min. Do not shake or vortex the tubes.

5.2.3 Centrifuge the tubes for a minimum of 2 min. (The colored aqueous layer should be on the bottom after centrifugation.)

5.3 Concentration

5.3.1 Insert the appropriate number of Disposable Concentration Cups into the wells of the OMEGA-12 Concentrator. With a disc-handling pin, place one TOXI-DISCS Blank A into each cup.

5.3.2 With a disposable transfer pipet, transfer the upper organic layer from each tube to the appropriate cup. Be careful not to transfer any of the lower (colored) aqueous layer. Note 10.1, 10.2.

5.3.3 Place the OMEGA-12 Concentrator on the electric warmer and cover with the OMEGA-12 screen. Evaporate the sample extracts with a gentle current of warm air from the heat gun directed across the top of the cups to speed evaporation. Evaporate to dryness. Perform evaporation in a well-ventilated area. Note 10.3.

5.4 Inoculation

5.4.1 Place a TOXI-GRAMS A on a clean, firm, flat surface.

5.4.2 With a disc-handling pin, insert the concentrated sample disc(s) into the center openings of the chromatogram. Hold the disc in place with a clean press card and remove the pin. Cover the disc end of the chromatogram with the card and press the discs into place by applying gentle pressure from the flat surface of a fingernail. Be careful not to damage the discs or the chromatogram. Each disc should fit snugly in the opening and in the plane of the chromatogram. Note 10.4.

5.4.3 Place the "loaded" chromatogram on the warmer, face up, with the disc ends slightly off the edge. Heat activate the chromatogram 30-60 s before development. Note 10.5.

5.5 Development

5.5.1 With a glass pipette or repipette, transfer 3 mL of stock developing solution into a small chromatography chamber. With a positive displacement pipet, transfer the recommended volume of ammonium hydroxide, as printed on the TOXI-GRAMS jar, into the chamber. Cap immediately and swirl vigorously for a few seconds. Note 10.6, 10.7.

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5.5.2 Remove the activated chromatogram from the warmer and lower, disc end first, into the chromatography chamber. Do not allow the side edges of the chromatogram to touch the walls of the chamber. Place cover on the chamber and do not disturb during migration.

5.5.3 Remove the chromatogram when the dye spots reach 9.5 cm (12-17 min), and place face down on the electric warmer for 30-60 s until the fumes have evaporated. Note 10.8, 10.9.

5.6 Detection

TOXI-GRAMS A are analyzed at four different stages. At each stage, the position (R_f), size, shape, and color characteristics of spots detected in the specimen channels are compared with the standard spots. The detection characteristics of the standard drugs are shown on Color Detection Guide A. See Section 6.0 for instructions on identification of unknown drug spots.

5.6.1 Preliminary Step
Place the chromatogram into TOXI-DIP A-1 for a minimum of 5 min, not to exceed 30 min (10-15 min is optimal). Several chromatograms may be placed in the A-1 jar and removed at intervals; however, both sides of an individual chromatogram must be sufficiently exposed to the vapor. Remove the chromatogram and place the lower two-thirds on the warmer for no more than 5 s to remove some of the fumes. Note 10.10.

5.6.2 Stage I
Dip the chromatogram slowly in and out of TOXI-DIP A-2; hold over the jar for 15-60 s, until the green center of pseudoephedrine (TOXI-DISCS A-2) develops. Observe specimen channels and compare with standard drug spots. Record observations on the TOXI-LAB A Worksheet. Note 10.11, 10.12.

5.6.3 Stage II
Dip the chromatogram in water once, quickly; hold over the jar for 3-5 s. Dip quickly once again. Allow the blue color of imipramine (TOXI-DISCS A-3) to fully develop, then continue dipping 2 to 3 times until morphine and codeine (TOXI-DISCS A-2) turn tan. Some drugs will become visible for the first time, others will fade, and still others will change color. Observe specimen channels and compare with standard drug spots. Record observations on the worksheet. Note 10.13.

5.6.4 Stage III
Lightly blot the chromatogram on a clean paper towel to remove excess water. Place the chromatogram in the Module III and observe the chromatogram over transmitted UV light (365 nm). Compare specimen drug spot fluorescence with standard drug spots and record observations. Note 10.14.

5.6.5 Stage IV
Dip the chromatogram in TOXI-DIP A-3 and leave immersed for at least 10 s. Remove the chromatogram from the jar and compare specimen drug spots with standard drug spots. Record observations. Note 10.15, 10.16, 10.17.

5.7 Use of Additional Drug Standards and Controls

5.7.1 TOXI-DISCS Drug Standards
In addition to drug standard discs on prestandardized chromatograms (TOXI-GRAMS A), additional drug standard discs are available for use with TOXI-LAB: TOXI-DISCS Library II Set, TOXI-DISCS A-1 through A-4, Special Procedure Standard Discs, Supplemental Standard Discs, and Special Standard Discs (custom formulated). These discs may be used for specific drugs in the initial drug screen, or to verify drugs detected in the initial drug screen.

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To use, simply insert the standard disc of choice into the chromatogram as per the inoculation instructions, Section 5.4. The discs may be inoculated into any of the six openings in TOXI-GRAMS Blank A, or into one of the two center openings of TOXI-GRAMS A.

5.7.2 TOXI-CONTROLS

TOXI-CONTROLS Nos. 2, 3, 4, 5, 6, and 19 are positive urine controls containing various drugs detected with TOXI-LAB A. These controls also contain drugs detected with TOXI-LAB B (barbiturates and other hypnotics), and TOXI-LAB THC (cannabinoids). TOXI-CONTROL No. 1 is a negative urine control.

TOXI-CONTROLS are designed for use as procedure controls (see Quality Control Procedure, Section 8.0). Throughout the procedure, the control should be treated the same as a urine specimen.

TOXI-CONTROLS should be used to control the TOXI-LAB procedure and materials. One procedural control per analyst per day is recommended.

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6.0 IDENTIFICATION

6.1 General Identification Procedure

6.1.1 Since most metabolites migrate to a lower position than the parent drug, observe the TOXI-GRAMS from the top downward.

6.1.2 Match position and color of any spot detected in the specimen channel with a drug standard, either included on TOXI-GRAMS A or available on other TOXI-DISCS drug standards, having the same characteristics at each stage of detection. (Specimen drug spot should be about the same size as the standard for comparison; use proper dilution, Note 10.2). **Identification is made when the specimen spot matches a standard in position and color in all four stages.**

6.1.3 Metabolites and other drugs will frequently be detected which do not match any of the TOXI-DISCS drug standards. Do not expect to identify each and every spot detected. Even an experienced analyst may be puzzled from time to time by unusual drugs and metabolites. However, TOXI-LAB includes teaching materials which will allow you to recognize many of these substances. The most important aid is the TOXI-LAB Drug Compendium, which pictures the great majority of commonly detected drugs and metabolites in the PHOTO-GRAMS section in ascending R_f. In studying the PHOTO-GRAMS, you will note that many drugs have highly specific characteristics and metabolite patterns, which are helpful in selecting appropriate standards for substance identification. The TOXI-LAB A Drug Characteristics Table includes a partial list of drugs detected with TOXI-LAB A, and should also be very helpful in selecting standards for the identification of drugs.

The TOXI-LAB Validation Procedure and TOXI-LAB Special Procedures may also be used to identify unknown drugs.

6.2 Use of the TOXI-LAB Drug Compendium

The TOXI-LAB Drug Compendium may be used to help select drug standards for the identification of unknown drug spots and/or metabolites detected in the specimen channel.

6.2.1 For each unknown drug spot, search the Compendium for drugs with similar R_f values.

6.2.2 Locate the drug and its R_f value in the Drug Index of the TOXI-LAB Drug Compendium.

6.2.3 Refer to the PHOTO-GRAMS A section of the TOXI-LAB Drug Compendium. Scan the PHOTO-GRAMS that are a full R_f above and below the selected standard spot. Compare unknown drug spot color and shape characteristics with those of the various drugs pictured in the PHOTO-GRAMS.

6.2.4 List all drugs from PHOTO-GRAMS that show similarities to the unknown drug spot through all four stages.

6.2.5 Choose a drug standard disc(s), containing those drugs in question, from the additional TOXI-DISCS drug standards (see Section 5.7.1). Reanalyze the specimen comparing the specimen drug spot with TOXI-DISCS drug standard spots. **Identification is made when the specimen spot matches a standard in position and color in all four stages.**

6.3 TOXI-LAB Validation Procedure

The TOXI-LAB Validation Procedure may be used, following initial detection with TOXI-LAB A, to increase the analyst's confidence level in the identification of basic and neutral drugs. The validation procedure is a rapid thin-layer chromatographic method, employing TOXI-GRAMS C_8 bonded phase chromatograms for the separation of drugs detected on TOXI-GRAMS A. See the TOXI-LAB Validation Procedure Manual for complete instructions.

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6.4 TOXI-LAB Special Procedures

TOXI-LAB Special Procedures may be used to enhance the detection of specific drugs by increasing detection specificity and/or sensitivity. See TOXI-LAB Special Procedures or the TOXI-TIPS Technical Information section of the TOXI-LAB Drug Compendium.

6.5 Identification Procedure — Discussion

The absolute position (R_f) of the standard spots may vary, within quality control guidelines, from those shown on the Detection Guides. However, the relative positions will be the same, e.g. codeine will always migrate above morphine. Some slight variation in color characteristics of the standard spots may also occur from one test to another. **Therefore, it is important to recognize that the Detection Guides are for orientation purposes only, and that the unknown spots must be matched to the standard spots on the actual chromatogram.**

Two different drugs may have similar migration and staining characteristics. However, careful attention to subtle detail will frequently allow a correct interpretation.

Initially, new users should learn to differentiate artifacts and trivia from significant drug spots. Almost every chromatogram may have some artifactual material immediately above the unknown disc and at the solvent front. Common rubber stopper artifacts are depicted in the PHOTO-GRAMS A section of the TOXI-LAB Drug Compendium. With urine, a pink artifact is frequently seen on TOXI-GRAMS A near methadone at Stage I only.

Patient information such as age, state of consciousness, drugs suspected, and drugs available can be very helpful to the analyst.

It is advisable to perform one or more tests on a urine control during initial testing to familiarize analysts with the procedure. Subsequent testing of controls may be established on a periodic basis.

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7.0 PREPARATION OF NON-URINE SPECIMENS

Urine is the single best specimen for analysis since the largest number of drugs and metabolites are found in this medium. However, other non-urine specimens may also be analyzed. Conduct the following procedures for the preparation of gastric fluid, serum or plasma, and non-biological materials (pills, powders, capsules, liquids) prior to TOXI-LAB A.

7.1 Gastric Fluid

7.1.1 Prior to analysis, filter the gastric specimen and dilute the filtrate at least 1:10.

7.1.2 Place 5 mL of dilute gastric filtrate into the extraction tube. Cap, and mix by inversion for 2 min.

7.1.3 Proceed to Section 5.2.3.

7.2 Serum or Plasma

7.2.1 Add 3 mL of water to a TOXI-TUBES A, cap, and mix for a few seconds.

7.2.2 Add 2 mL of serum or plasma to the same tube, cap, and mix by inversion for 2 min.

7.2.3 Proceed to Section 5.2.3.

NOTE: Drugs which characteristically have a low plasma concentration, such as morphine, will not be detected. Additionally, a lipid artifact seen at Stages I to IV between R_f 0.8 and 1.0 will obscure certain high migrating drugs such as diazepam and methaqualone. A smaller lipid artifact may also be present in the region below methamphetamine-amphetamine. A preliminary migration can remove much of the lipid; refer to "Serum Clean-Up" in the TOXI-TIPS section of the TOXI-LAB Drug Compendium.

7.3 Nonbiological Materials (pills, powders, capsules, liquids)

7.3.1 Crush pills to a fine powder using a clean mortar and pestle.

7.3.2 Add 2 to 3 mg of powder material or 2 to 3 µL of liquid material to a TOXI-TUBES A. Add deionized (or distilled) water to the 5 mL arrow. Cap and mix by inversion for 2 min.

7.3.3 Centrifuge the tube for 2-5 min.

7.3.4 Place a TOXI-DISCS Blank A into each of two concentration cups in the OMEGA-12.

7.3.5 Transfer 2 to 3 drops of the A extract to one cup and approximately 20 drops to the other cup. Save the remainder of the extract in the tube.

7.3.6 Proceed with Section 5.3.3.

NOTE: This technique will bracket most drugs except for those in extremely low concentrations. In this case the remainder of the extract may be processed or the sample size increased.

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8.0 QUALITY CONTROL PROCEDURE

The following procedure outlines acceptance/rejection criteria for variables in a TOXI-LAB A analytical run. An analytical run in this procedure is defined as all specimens run by a specific individual during a particular day.

8.1 Extraction Criteria
To control extraction, each operator should use the following procedure to demonstrate proper extraction of a urine control such as TOXI-CONTROL No. 19.

8.1.1 Analyze a urine control through the extraction process for every lot of TOXI-TUBES A used. (Extraction efficiency is consistent within a given lot of TOXI-TUBES A; Appendix II includes data used to validate this consistency.)

8.1.2 Record the lot number of TOXI-TUBES A used for each analytical run.

8.1.3 Analyze a urine control with every analytical run.

8.2 Inoculation Criteria
The inoculation process is controlled by proper disc fit. For consistent and proper placement of the disc, inspect the inoculated chromatogram and ensure that the following criteria are met.

8.2.1 The disc is in the plane of the chromatogram (i.e. lies flush with the chromatogram surface).

8.2.2 There are no spaces between the disc and chromatogram.

8.2.3 Tap the chromatogram against a firm surface. If the disc is firmly in place, it will not be dislodged.

8.3 Development Criteria
To control the migration of standards and unknowns, ensure that the following development criteria are met.

8.3.1 Pseudoephedrine: R_f is 0.15 $\pm$0.03. The spot does not overlap methamphetamine.

8.3.2 Diazepam, Cocaine, Acetaminophen: Separate detection of the spots in this order at Stage IV.

8.4 Detection Criteria
To control the visualization of standards and unknowns, ensure that the following detection criteria are met.

8.4.1 Stage I
Pseudoephedrine: A yellow spot comparable in size to the other standards forms within 5-10 s; a green center forms within 30-60 s.

8.4.2 Stage II
Imipramine: A persistent blue spot comparable in size to the other standards forms after 1 to 2 water washes.

8.4.3 Stage III

8.4.3.1 Pseudoephedrine: The spot exhibits strong blue fluorescence.

8.4.3.2 Imipramine: The spot exhibits dull bluish-green fluorescence.

8.4.4 Stage IV
Caffeine: A grey spot comparable in size to the other standards.

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8.5 Correction Procedure

TOXI-LAB A extraction, inoculation, and concentration are easily controlled procedures that yield consistent results when properly followed. When out-of-control condition exists, correction is straightforward.

Troubleshooting is more difficult in the development and detection of chromatograms. When out-of-control conditions exist, the TOXI-LAB A procedure should be reviewed and Troubleshooting, Section 9.0, should be used as needed to make corrections.

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9.0 TROUBLESHOOTING

PROBLEMS	PROBABLE CAUSES	RECOMMENDATIONS
LOW MIGRATION	Weak ammonium hydroxide	• Use concentrated 56% ammonium hydroxide (28% ammonia). • Buy pint bottles of ammonium hydroxide or dispense into pint containers. Pipet from working reagent vial: change daily or as needed. • Accurately measure the ammonium hydroxide required (refer to the TOXI-GRAMS jar label). • Add ammonium hydroxide to developing solution immediately prior to migration.
	Insufficient amount of ammonium hydroxide	• Add more ammonium hydroxide to the developing solution.
	Incorrect developing solvent	• Place TOXI-GRAMS into A developing solution.
HIGH MIGRATION	Temperature >80°F or 27°C High humidity	• Refrigerate developing solvent. • Heat-activate the chromatograms for 2 to 3 min prior to development. • Develop the chromatogram in cool area of laboratory away from any heat source.
	Too much ammonium hydroxide	• Decrease the volume of ammonium hydroxide in the developing solution.
	Contaminated ethyl acetate (A developing solvent)	• Check or replace A developing solvent.
	Overdevelopment	• Remove the chromatogram from developing solvent when pink dye markers are between the 9- and 10-cm line.
STREAKY MIGRATION	High humidity	• Heat-activate the chromatograms for 2 to 3 min prior to development.
	Improper disc fit	• Fit the disc correctly in the plane of the TOXI-GRAMS. No light should be visible surrounding it.
	High concentration of drugs	• Dilute the specimen if concentration is excessive.
STREAKY MIGRATION OF SYMPATHOMIMETIC AMINES	Contaminated ethyl acetate	• Use uncontaminated ethyl acetate or Ethyl Acetate TOXI-LAB Grade.
SKEWED MIGRATION	Chromatogram touching sides of chromatography chamber	• Center the chromatogram in the chamber; the bottom corners of the chromatogram should not touch the side walls of the chamber.
	Heat source near chromatography chamber	• Develop the chromatogram away from the warmer or other heat source.

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	Failure to mix ammonium hydroxide with developing solution	• Thoroughly mix the ammonium hydroxide with the developing fluid.
CONTAMINATION	Spattering during concentration	• Prevent spattering by beginning concentration step with a cool OMEGA-12.
	Contaminated press card	• Dipose of cards used to press sample discs in place on TOXI-GRAMS after each use.
	Improper handling of chromatograms	• Handle the chromatogram at or above the 10-cm line to the left or right of the patient zone with clean forceps to avoid artifacts.
	Improper caps on reagent jars	• Use caps supplied with the system; avoid interchanging.
NON-VISUALIZATION Unknown Sample	Contaminated disc	• Do not contaminate sample disc with lower aqueous layer from the TOXI-TUBES.
	Excessive heat applied to disc during evaporation or after development of chromatogram	• Remove the unknown disc from the OMEGA-12 as soon as it is dry. Heat the TOXI-GRAMS minimally after development.
	Excessive delay between detection stages	• Perform detection steps with a minimal delay between stages. Lightly blot the chromatogram with a paper towel after the water dip.
	Low solvent volume in TOXI-TUBES	• Do not use TOXI-TUBES if solvent is below the arrow on the tube.
	Insufficient mixing time with TOXI-TUBES	• Mix TOXI-TUBES gently by inversion for 2 min.
	Insufficient specimen	• Refer to Sections 3.4, 5.2.1, and 7.0 for correct specimen volume and handling.
Standards	Reagent deterioration	• Make fresh reagents; use proper caps; keep reagent jars full and covered.

STAGE I PROBLEMS	PROBABLE CAUSES	RECOMMENDATIONS
Meperidine not detected Opiates demonstrate atypical colors Sympathomimetic amines do not develop typically colored centers	Weak formaldehyde Improper exposure to formaldehyde	• Use fresh 37% formaldehyde; change weekly. • Expose both sides of the chromatogram to formaldehyde for a minimum of 5 min, not to exceed 30 min.

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Methadone appears faded or gray Phenothiazines appear pink	Contaminated sulfuric acid (A-2 reagent)	• Use only fresh concentrated sulfuric acid (95-98% or 36 normal) • Keep working reagent (A-2 jar) full at all times so little air is trapped. • Heat off lower two-thirds of chromatogram prior to dipping in sulfuric acid (TOXI-DIP A-2).
STAGE II PROBLEMS	**PROBABLE CAUSES**	**RECOMMENDATIONS**
Phenothiazines (pink color) and/or imipramine (blue color) slow to appear	Acidic water	• Use deionized water. Change water daily or after every 5-10 chromatograms.
Small phenothiazine standards and/or improper color formation of imipramine	Improper washing of chromatogram	• Dip chromatogram once into and out of water; hold and wait 3 to 5 s. Dip once again, hold and wait for phenothiazines to turn pink and imipramine to turn blue; continue to water wash 2 to 3 times until opiates are tan.
Imipramine fails to appear	Plasticizers contaminating developing fluid	• Do not use rubber- or plastic-capped containers when formulating the A developing fluid.
STAGE III PROBLEMS	**PROBABLE CAUSES**	**RECOMMENDATIONS**
Atypical fluorescence	Week formaldehyde	• Use fresh 37% formaldehyde.
	Improper timing during detection process	• Wait for meperidine to appear at Stage I before proceeding to Stage II.
	Improper washing of chromatogram	• Dip the chromatogram once into and out of the water, hold and wait for phenothiazines to turn pink; continue to water wash the chromatogram 2 to 3 times until opiates are tan.
	Use of improper UV light	• Use 365nm long-wave UV light.
	Observed with reflected UV light	• View chromatogram with transmitted UV light. • View chromatogram in completely darkened environment.
STAGE IV PROBLEMS	**PROBABLE CAUSES**	**RECOMMENDATIONS**
Meprobamate not detected	Weak or poor exposure to formaldehyde	• Use fresh 37% formaldehyde; expose both sides of the chromatogram for a minimum of 5 min; do not heat off upper one-third of chromatogram before dipping into sulfuric acid (A-2 reagent).
Cocaine and acetaminophen do not separate	Weak ammonium hydroxide	• Use fresh, concentrated (58%) ammonium hydroxide.

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	Insufficient amount of ammonium hydroxide	• Add more ammonium hydroxide to the developing solution.
Diazepam and cocaine do not separate	Temperature >80°F or 27°C High humidity	• Refrigerate developing solvent. • Heat-activate the chromatograms for 2 to 3 min prior to development. • Develop the chromatogram in cool area of laboratory away from any heat source.
	Too much ammonium hydroxide	• Decrease the volume of ammonium hydroxide.
Atypical staining Caffeine does not have proper color formation	Deteriorated A-3 reagent (modified Dragendorff's)	• Make fresh Dragendorff's (A-3).

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10.0 TECHNICAL NOTES

10.1 To avoid dripping, hold the pipet vertically during transfer. Contamination from the lower aqueous layer may inhibit drug adsorption and will show as effervescence from the sample disc when dipping in TOXI-DIP A-2.

10.2 Drug spots in high concentration may be too large for correct interpretation. The following technique will allow concurrent analysis of an undiluted and a diluted specimen (approximately 1:20) and is especially recommended for suspected overdose cases.

Extract the undiluted specimen in the usual manner. For each extraction tube, place 2 TOXI-DISCS Blank A into separate concentration cups in the OMEGA-12. Concentrate 5 drops of the extract onto one of the discs and the remainder of the extract onto the second disc. Fit the dried discs into the center openings of a TOXI-GRAMS A. Develop and detect in the usual manner. If concentration is still too high, the specimen may require dilution prior to extraction.

10.3 In order to minimize loss of certain volatile drugs, the discs should be removed from the evaporation cups immediately after drying (discs will blanch when completely dry).

10.4 The second opening in the TOXI-GRAMS may be used for a second specimen, quality control, an additional TOXI-DISCS drug standard, special disc, or two different concentrations of the same specimen (see Note 10.2).

10.5 High or streaky migration of drug spots may occur if the TOXI-GRAMS are not heated (activated) for 30-60 s prior to developing.

10.6 Migration of basic drugs is strongly influenced by the concentration of ammonium hydroxide in the developing fluid. With TOXI-GRAMS A, increasing the concentration of ammonia increases migration. (2 to 3 mL of concentrated ammonium hydroxide should be stored in a small **tightly capped** vial and dispensed as needed. Replace with fresh ammonium hydroxide weekly or more frequently if needed.)

10.7 To prevent high migration, development should be done in a cool area of the laboratory, never in direct sunlight or close to a source of heat such as the warmer.

10.8 Excessive or prolonged heating of TOXI-GRAMS or TOXI-DISCS should be avoided as loss of heat-labile drugs such as amphetamines may occur. TOXI-DISCS A have been treated to inhibit volatization of some drugs.

10.9 The 3-mL aliquot of ammoniated developing solvent can be used for only one test. It is not necessary to wash the chromatogram chambers between each analysis, simply invert to drain.

10.10 Detection of several drugs such meperidine and meprobamate depends on some formaldehyde remaining on the chromatogram. Leave the top one-third of the chromatogram off the electric warmer for good detection of meprobamate.

10.11 Waiting longer than a minute between the TOXI-LAB A detection stages may result in reduced visualization of such drugs as cocaine, nicotine, and caffeine.

10.12 Fluorescent lighting is recommended for viewing TOXI-GRAMS A at Stage I.

10.13 At Stage II, an additional dip or two in water may be required to produce properly colored drug spots such as trifluoperazine, imipramine, chlorpromazine, triflupromazine, and trimeprazine (TOXI-DISCS A-3).

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10.14 At Stage III, a certain degree of non-specific fluoresence may be detected from most urine specimens.

10.15 TOXI-GRAMS A may be photocopied following Stage IV. Place in a plastic sleeve prior to photocopying.

10.16 Spots on TOXI-GRAMS A at Stage IV can be preserved by placing the chromatogram, drained free of excess A-3 reagent, in a screw-cap jar containing a few grams of iodine crystals.

10.17 For best results, perform the entire procedure without interruption. If it is necessary to stop, do so after any of the following steps; however, do not wait longer than 24 hours to complete the procedure.

— After adding the sample to the TOXI-TUBES A and mixing.

— After centrifuging.

— After concentration of the sample extract. (Sample discs may be placed in small labeled test tubes, protected from light.)

— After the chromatogram has been developed. (Place the chromatogram in a desiccator, protected from light.)

Stopping for an extended period of time is not recommended, as increased artifacts or drug breakdown may occur.

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11.0 LIMITATIONS OF THE METHOD

Using 5 mL of urine, the threshold of detection for most drugs is approximately 1.0 µg or less per mL of urine. (Drug detection limits are included in the Drug Information Section of the TOXI-LAB Drug Compendium, however, it is recommended each laboratory establish its own detection limits.) Some substances may not be detected with TOXI-LAB, and a negative result does not mean that all drugs are absent. Questionable findings should be confirmed by employing a different methodology.

There is the possibility that other substances and/or factors not listed above may interfere with the test, for example, technical or procedural errors.

NOTE: Substitutions of other materials and/or deviations from the Instruction Manual could yield less than optimum results and as a consequence may void the warranty on this product.

11.1 Expected Values
Depending upon circumstances, the level of drug in a given specimen will vary considerably—depending upon such things as dose, route of administration, half-life, and time of sampling.

11.2 Performance Characteristics

11.2.1 Accuracy
Urine specimens with known concentrations of drugs, listed in the table below, were analyzed according to the TOXI-LAB A Drug Detection System Procedure and were shown to be positive.

11.2.2 Precision
The following drugs of abuse were evaluated. In each case a negative urine was spiked with the drug at the concentration shown. The sample was then aliquoted and 10 aliquots of each were tested with the TOXI-LAB A Drug Detection System.

SUMMARY OF DATA

Drug Name	Concentration mL	Mean Rf	±Rf	Rf Range
Amphetamine	0.5	0.31	0.010	0.30-0.33
Methamphetamine	0.5	0.24	0.007	0.23-0.25
Diazepam	1.0	0.87	0.005	0.86-0.88
Nordiazepam	1.0	0.84	0.007	0.83-0.85
Phencyclidine	0.5	0.86	0.006	0.85-0.87
Benzoylecgonine	1.0	0.30	0.007	0.28-0.31
Codeine	0.5	0.24	0.006	0.23-0.25
Morphine	1.0	0.14	0.005	0.14-0.15
Methaqualone	1.0	0.88	0.006	0.87-0.89
Methadone	0.5	0.67	0.015	0.67-0.70

11.2.3 Sensitivity
When used as directed, the TOXI-LAB A Drug Detection System is sensitive to 1 µg/mL for most drugs and drug classes.

11.2.4 Specificity
A unique identification is made when a drug and/or its metabolite is detected in the unknown zone and matches an adjacent standard in position (Rf), size, shape, and color through all four stages.

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APPENDIX I

TOXI-GRAMS A DRUG STANDARDS

DISC	Generic Name	Brand Name	Use	Class	Reaction
A-1	Propoxyphene	DARVON®	Analgesic	Narcotic	Basic
	Methadone	DOLOPHINE®	Analgesic	Narcotic	Basic
	Meperidine	DEMEROL®	Analgesic	Narcotic	Basic
	Codeine	—	Analgesic	Narcotic	Basic
	Morphine	—	Analgesic	Narcotic	Basic
A-2	Diazepam	VALIUM®	Tranquilizer	Benzodiazepine	Neutral
	Cocaine	—	Stimulant	Alkaloid	Basic
	Acetaminophen	TYLENOL®	Analgesic	Nonnarcotic	Acidic
	Caffeine	—	Stimulant	Alkaloid	Basic
	Nicotine	—	Stimulant	Alkaloid	Basic
	Amphetamine	BENZEDRINE®	Stimulant	Symp. amine	Basic
	Methamphetamine	DESOXYN®	Stimulant	Symp. amine	Basic
	Pseudoephedrine	SUDAFED®	Decongestant	Symp. amine	Basic
A-3	Phencyclidine (PCP)	—	Hallucinogen	—	Basic
	Trimeprazine	TEMARIL®	Antihistamine	Phenothiazine	Basic
	Triflupromazine	VESPRIN®	Tranquilizer	Phenothiazine	Basic
	Chlorpromazine	THORAZINE®	Tranquilizer	Phenothiazine	Basic
	Imipramine	TOFRANIL®	Antidepressant	Tricyclic	Basic
	Trifluoperazine	STELAZINE®	Tranquilizer	Phenothiazine	Basic
	Quinine	—	Adulterant/ Antimalarial	Alkaloid	Basic
A-4	Methaqualone	QUAALUDE®	Hypnotic	Nonbarbiturate	Basic
	Meprobamate	MILTOWN®, etc.	Tranquilizer	Carbamate	Neutral
	Amitriptyline	ELAVIL®	Antidepressant	Tricyclic	Basic
	Doxepin	SINEQUAN®	Antidepressant	Tricyclic	Basic
	Nortriptyline	AVENTYL®	Antidepressant	Tricyclic	Basic
	Strychnine	—	Stimulant	Alkaloid	Basic

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APPENDIX II

TOXI-LAB, Inc. TOXI-TUBES A Intra-lot Consistency Data

Intra-lot consistency is checked by the manufacturer on every batch of TOXI-TUBES A with a reference and minimum detection urine controls containing the following drugs.

Drug	Concentration of Minimum Detection Urine Control (µg/mL)	Concentration of Reference Urine Control (µg/mL)
Amphetamine	1.0	3.0
Imipramine	0.5	1.5
Methadone	0.5	1.5
Morphine	1.0	3.0
Propoxyphene	1.0	4.0

Tube lots are not approved unless sample chromatograms show all drugs present in each detection stage. For every sample tube tested, a control tube from a previously approved lot is run simultaneously.

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2.2.2: Toxi-Lab Toxi-B Drug Dectection System (2.2.2.1 Toxi Lab Toxi-B Instruction Manual)

Orv rev. 0

TOXI-LAB® B DRUG DETECTION SYSTEM

INSTRUCTION MANUAL

Cat. No. 181B

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TOXI-LAB, Inc.
Irvine, CA 92718

TOXI-LAB® B DRUG DETECTION SYSTEM INSTRUCTION MANUAL

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1.0 INTRODUCTION

1.1 Description and Intended Use

TOXI-LAB® B Drug Detection System is a rapid thin-layer chromatographic method for the separation, detection and identification of barbiturates and other hypnotics (acidic and neutral drugs). TOXI-LAB B may be used by hospital, outpatient, reference, and forensic laboratories to screen for the presence of drugs in biological fluids and other samples in solid or liquid form.

For forensic testing purposes (i.e., drugs of abuse testing), the TOXI-LAB B Drug Detection System provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgement should be applied to any test result, particularly when preliminary positive results are used.

The TOXI-LAB AB Initial System includes materials required for TOXI-LAB A and TOXI-LAB B. This manual discusses the materials and procedures required for TOXI-LAB B only. For information on TOXI-LAB A, refer to the TOXI-LAB A Drug Detection System Instruction Manual.

1.2 Principle

TOXI-LAB B is a modification of the thin-layer chromatography (TLC) technique; the procedural steps of extraction, concentration, inoculation, development, and detection have been simplified for convenience, and to minimize sample processing time.

Drugs are extracted from biological fluids and other materials using TOXI-TUBES® B containing a mixture of solvents and buffering salts that extract acidic and neutral drugs. The solvent extracts are concentrated by heat and evaporation, depositing the unknown drugs onto discs of chromatographic media. The dried discs are then inserted (inoculated) into the center openings of TOXI-GRAMS® B.

The "loaded" chromatograms are developed by placing them in developing chambers containing small volumes of organic solvent. Elution of the unknown and standard drugs from the discs and the resulting position (R_f) of these drugs on the chromatograms occur during migration of the developing solvent. Detection of the unknown and standard drug spots is achieved when the chromatograms are dipped into chromogenic reagents. Identification is based on matching a drug spot in the unknown zone with an adjacent standard drug spot having the same R_f, size, shape, and color characteristics.

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2.0 REAGENTS AND MATERIALS

2.1 Materials Supplied in TOXI-LAB B

2.1.1 TOXI-KIT B-50 (Cat. No. 101B-50)

2.1.1.1 TOXI-GRAMS B-50 (Cat. No. 106B-50)
Drug-standardized 6-channel chromatograms for separation of acidic and neutral drugs; made of glass microfiber paper and impregnated with silicic acid. TOXI-DISCS® B-1, B-2, B-3, and B-4, impregnated with a total of 11 drugs and a dye marker, are pre-inserted into four of the six openings. See Appendix I for drug standards.

2.1.1.2 TOXI-TUBES B-50 (Cat. No 109B-50)
Tubes for extraction of acidic and neutral drugs; contain a mixture of solvents, buffering salts, and a phase-marking dye.

2.1.1.3 TOXI-DISCS Blank B-100 (Cat. No. 108B-100)
Discs for concentration of drugs; made of glass microfiber paper and silicic acid.

2.1.1.4 Chemicals for Preparation of TOXI-DIP® B Reagents (Cat. No. 111B)
Solutions for making TOXI-DIP B Reagents:

1 vial (3 mL/vial) s-diphenylcarbazone solution for making TOXI-DIP B-1 Reagent.

1 vial (3 mL/vial) silver nitrate solution for making TOXI-DIP B-2 Reagent.

1 vial (3.5 mL/vial) mercuric nitrate solution for making TOXI-DIP B-3 Reagent.

2.1.1.5 TOXI-LAB B Worksheets (Cat. No. 187B)
Worksheets showing TOXI-GRAMS B detection stages and standards; for recording observations. 50 sheets/pad.

2.1.2 TOXI-GRAMS Blank B-50 (Cat. No. 120B-50)
Nonstandardized 6-channel chromatograms for separation of acidic and neutral drugs; made of glass microfiber paper and impregnated with silicic acid. (For use with TOXI-DISCS drug standards.)

2.1.3 TOXI-CONTROL® Six Pack (Cat. No. 170G)*
Package of 6 urine controls (1 negative, 5 positives); contains 1 vial each of TOXI-CONTROL Nos. 1, 2, 3, 4, 5, and 6; 5 mL/vial.

2.1.4 TOXI-CONTROL No. 19 (Cat. No. 191AB)*
Positive urine control; 50 mL/vial.

2.1.5 Ethyl Acetate TOXI-LAB Grade (Cat. No. 202)*
Pretested ethyl acetate for use with TOXI-LAB.

2.2 Equipment and Accessories Supplied in TOXI-LAB B

2.2.1 TOXI-LAB Workstation (Cat. No. 150)*

2.2.1.1 Workstation Module I (Cat. No. 151)
Work center for sample preparation; includes Storage Tray, Heat Gun, and Heat Gun Holder.

2.2.1.2 Workstation Module II (Cat. No. 154)
Work center for drug detection; includes turntable and dipping jars with special caps. (Also includes extra storage jar cap and friction band.)

*Items common to TOXI-LAB A and TOXI-LAB B.

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2.2.1.3 Workstation Module III (Cat. No. 155)
Work center for viewing TOXI-GRAMS with ultraviolet light.

2.2.1.4 OMEGA-12 Extraction Solvent Concentrator with Screen (Cat. No. 153)
Solvent concentrator with 12 wells for Disposable Concentration Cups, and screen cover.

2.2.1.5 Disposable Concentration Cups (Cat. No. 152)
Disposable aluminum cups for evaporation of extraction solvents.

2.2.2 Electric Warmer with Protective Sheet (Cat. No. 118)*
Low-heat source for solvent evaporation.

2.2.3 Ultraviolet Light (Cat. No. 116)*
UVL-21 Blak-Ray, long-wave UV light (365 nm) for viewing TOXI-GRAMS.

2.2.4 Microdispenser (Cat. No. 190L)*
Drummond Dialamatic Microdispenser; positive displacement pipet with variable volume to 50.0 μL.

2.2.5 TOXI-RACK® 3 Set (Cat. No. 113C)*
Multiple development racks for developing up to three TOXI-GRAMS simultaneously; includes Small Chromatography Jars with Cap.

2.2.6 Forceps (Cat. No. 184)*
Stainless steel forceps for handling TOXI-GRAMS.

2.2.7 Disc-handling Pins (Cat. No. 186)*
Pearl-headed pins for handling TOXI-DISCS.

2.2.8 Chromatogram Holder (Cat. No. 163)*
Clip for holding chromatograms while dipping.

2.2.9 Ammonium Hydroxide Vial with Cap*
Small amber vial for storing small volumes of ammonium hydroxide.

2.2.10 Small Chromatography Jar with Cap (Cat. No. 113A)*
Glass chamber for development of TOXI-GRAMS.

2.2.11 TOXI-DIP B-1 Stock Bottle (Cat. No. 119B)
Labeled, 500-mL capacity bottle for storing TOXI-DIP B-1 Reagent stock solution.

2.2.12 TOXI-DIP B-2 Stock Bottle (Cat. No. 119D)
Labeled, 500-mL capacity bottle for storing TOXI-DIP B-2 Reagent stock solution.

2.2.13 TOXI-DIP B-3 Stock Bottle (Cat. No. 119E)
Labeled, 500-mL capacity bottle for storing TOXI-DIP B-3 Reagent stock solution.

2.2.14 TOXI-LAB B Developing Solution Bottle (Cat. 112B)
Labeled, 100-mL capacity bottle for storing TOXI-LAB B Developing Solution.

2.3 Reference and Educational Aids Supplied in TOXI-LAB B

2.3.1 TOXI-LAB B Drug Detection System Instruction Manual (Cat. No. 181B)
Instruction manual for materials and procedures involved in the TOXI-LAB B Drug Detection System.

2.3.2 Color Detection Guide B (Cat. No. 182B)
Color photograph showing TOXI-GRAMS B drug standards in two detection stages.

*Items common to TOXI-LAB A and TOXI-LAB B.

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2.3.3 TOXI-LAB Drug Compendium (Cat. No. 201)*
Compendium of drug and pharmaceutical information, full-color PHOTO-GRAMS® showing detection characteristics of drugs and metabolites, and TOXI-TIPS® Technical Information and Procedures.

2.3.4 TOXI-LAB AB Training Video Tape (Cat. No. 230)*
VHS video tape of the TOXI-LAB B Procedure; also includes demonstrations of the TOXI-LAB A Procedure, TOXI-LAB Validation Procedure, and fundamentals of troubleshooting.

2.4 Reagents Required for TOXI-LAB B

2.4.1 Ethyl acetate TOXI-LAB Grade (Cat. No. 202) or equivalent.*

2.4.2 Ammonium hydroxide, approximately 58% NH_4OH, or 28% to 30% NH_3, reagent grade.*

2.4.3 Sulfuric acid, concentrated, reagent grade.*

2.4.4 Dichloromethane, reagent grade.

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*Items common to TOXI-LAB A and TOXI-LAB B.

3.0 PROPER HANDLING AND STORAGE OF MATERIALS AND SPECIMENS

3.1 Chemicals and Reagents

Read labels and adhere to cautions and warnings. Follow instructions for reagent preparation and storage, Section 4.0.

3.2 TOXI-GRAMS

TOXI-GRAMS are fragile and susceptible to contamination. Handle carefully with clean forceps.

Store standardized TOXI-GRAMS at 15°-30°C in the desiccant-containing jars provided. If the desiccating crystals turn pink, remove them and reactivate by heating at approximately 100°C until they turn blue, then return them to the jar. Adsorption of moisture may cause poor resolution and/or deterioration of the standard drugs. DO NOT USE if this is observed when chromatograms are tested (see Section 8.0, Quality Control Procedure). Desiccation is not necessary for nonstandardized (blank) chromatograms.

3.3 TOXI-DISCS

Use disc-handling pins (touching the discs lightly) and clean press cards when handling TOXI-DISCS. Strips of index card material, or similar paper material, may be used as press cards.

Store TOXI-DISCS drug standards at 15°-30°C in the desiccant-containing vials provided. If the desiccating crystals turn pink, remove them and reactivate by heating at approximately 100°C until they turn blue, then return them to the vial. Test the discs prior to use; adsorption of moisture may cause drug deterioration. DO NOT USE if this is observed when discs are tested.

3.4 Specimens

Avoid specimen contact with rubber objects or soft plastics; some rubber stoppers may produce artifacts with TOXI-LAB B (see "Artifact" in TOXI-TIPS Technical Information section of the TOXI-LAB Drug Compendium). Follow safe laboratory procedures and precautions for handling biological hazards.

Biological specimens should be refrigerated if the analysis is not to be performed immediately. See Section 7.0 for preparation of non-urine specimens.

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4.0 REAGENT PREPARATION, PRECAUTIONS, AND STORAGE INSTRUCTIONS

4.1 TOXI-DIP B-1 Reagent (diphenylcarbazone solution)
Empty **completely** the contents of one B-1 chemical vial into the B-1 jar. Fill with dichloromethane to approximately 1/4 inch from the top of the jar (final volume approximately 250 mL). Cap **tightly** and mix. Store at room temperature. As reagent is used, replenish from stock. Use with adequate ventilation and dispose of properly.

4.2 TOXI-DIP B-2 Reagent (silver nitrate solution)
Empty the contents of one B-2 chemical solution vial into the B-2 jar. Fill with deionized (or distilled) water to approximately 1/4 inch from the top of the jar (final volume approximately 250 mL). Cap **tightly** and mix. Store at room temperature. As reagent is used, replenish from stock and dispose of properly.

4.3 TOXI-DIP B-3 Reagent (mercuric sulfate solution)
Empty completely the contents of one B-3 chemical vial into the B-3 jar. Fill to approximately 3/4 full with deionized (or distilled) water. **Cautiously** and with stirring, add 10 mL concentrated sulfuric acid. Dilute with deionized water to approximately 1/4 inch from the top of the jar. Cap **tightly** and mix. Store at room temperature. As reagent is used, replenish from stock. Use with adequate ventilation and dispose of properly.

4.4 Stock Developing Solution
To the developing solution bottle add 60 mL of dichloromethane and 40 mL of ethyl acetate. Cap **tightly** and mix well. Store at room temperature. (A larger volume may be prepared in a suitable container, and may be stored in a dispenser capable of delivering 3-mL aliquots.)

Working solution for development, with ammonium hydroxide added, is formulated immediately before use (see Section 5.5.1). Avoid the use of plastic pipets, containers, and stoppers when preparing, storing and dispensing developing solution. Ethyl acetate may deteriorate with time. Use with adequate ventilation and dispose of properly.

NOTE: Shelf life of detection reagents depends partly on frequency of use (number of chromatograms dipped into them). Detection of drug standards, as per Color Detection Guide B, indicates potent reagents. If poor detection is noted, replace reagents. See Quality Control Procedure, Section 8.0.

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5.0 TOXI-LAB B GENERAL PROCEDURE — URINE SPECIMENS

5.1 Preliminary Steps

5.1.1 Plug in the electric warmer.

5.1.2 Obtain the appropriate number of TOXI-TUBES B, TOXI-LAB B Worksheets, the Omega-12 Extraction Solvent Concentrator with Screen, and Disposable Concentration Cups. Label each with the respective sample numbers.

5.1.3 Allow specimens to reach room temperature before extraction.

5.2 Extraction

5.2.1 To TOXI-TUBES B, add the urine specimens up to the arrows indicating 4.5 mL. See Section 7.0 for the preparation of non-urine specimens.

5.2.2 Cap the tubes tightly and mix well by gentle inversion for a minimum of 2 min. Do not shake or vortex the tubes.

5.2.3 Centrifuge the tubes for a minimum of 2 min. (The colored aqueous layer should be on the bottom after centrifugation.)

5.3 Concentration

5.3.1 Insert the appropriate number of Disposable Concentration Cups into the wells of the OMEGA-12 Concentrator. With a disc-handling pin, place one TOXI-DISCS Blank B into each cup.

5.3.2 With a disposable transfer pipet, transfer the upper organic layer from each tube to the appropriate cup. Be careful not to transfer any of the lower (colored) aqueous layer. Note 10.1, 10.2.

5.3.3 Place the OMEGA-12 Concentrator on the electric warmer and cover with the OMEGA-12 screen. Evaporate the sample extracts with a gentle current of warm air from the heat gun directed across the top of the cups to speed evaporation. Evaporate to dryness. Perform evaporation in a well-ventilated area. Note 10.3.

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5.4 Inoculation

5.4.1 Place a TOXI-GRAMS B on a clean, firm, flat surface.

5.4.2 With a disc-handling pin, insert the concentrated sample disc(s) into the center openings of the chromatogram. Hold the disc in place with a clean press card and remove the pin. Cover the disc end of the chromatogram with the card and press the discs into place by applying gentle pressure from the flat surface of a fingernail. Be careful not to damage the discs or the chromatogram. Each disc should fit snugly in the opening and in the plane of the chromatogram. Note 10.4.

5.4.3 Place the "loaded" chromatogram on the warmer, face up, with the disc ends slightly off the edge. Heat activate the chromatogram 30-60 s before development. Note 10.5.

5.5 Development

5.5.1 With a glass pipette or repipette, transfer 3 mL of stock developing solution into a small chromatography chamber. With a positive displacement pipet, transfer the recommended volume of ammonium hydroxide, as printed on the TOXI-GRAMS jar, into the chamber. Cap immediately and swirl vigorously for a few seconds. Note 10.6, 10.7.

5.5.2 Remove the activated chromatogram from the warmer and lower, disc end first, into the chromatography chamber. Do not allow the side edges of the chromatogram to touch the walls of the chamber. Place cover on the chamber and do not disturb during migration.

5.5.3 Remove the chromatogram when the dye spots reach 9.5 cm (12-17 min), and place face down on the electric warmer for 30-60 s until the fumes have evaporated. Note 10.8, 10.9.

5.6 Detection

TOXI-GRAMS B are analyzed at two different stages. At each stage, the position (R_f), size, shape, and color characteristics of spots detected in the specimen channels are compared with the standard spots. The detection characteristics of the standard drugs are shown on Color Detection Guide B. See Section 6.0 for instructions on identification of unknown drug spots.

5.6.1 Preliminary Step
Dip the chromatogram once, **quickly**, in and out of TOXI-DIP B-1. Place the chromatogram in the drying rack until all of the dichloromethane has evaporated. **Do not** heat dry the chromatograms on the warmer. Note 10.10.

5.6.2 Stage I
Remove the caps from the TOXI-DIP B-2 and B-3 jars. Dip the chromatogram into TOXI-DIP B-2 and wait only momentarily; a golden-brown background will develop. Then **immediately** transfer the chromatogram into TOXI-DIP B-3 and agitate until the background clears.

It is important to observe color reactions in both TOXI-DIP B-2 and B-3. Record observations on the TOXI-LAB B Worksheet.

5.6.3 Stage II
Lightly blot the chromatogram on a clean paper towel to remove excess reagent. Place the chromatogram in the Module III and observe the chromatogram over transmitted UV light (365 nm). Compare specimen drug spot fluorescence or absorbance with standard drug spots and record observations. Note 10.11, 10.12, 10.13.

5.7 Use of Additional Drug Standards and Controls

5.7.1 TOXI-DISCS Drug Standards
In addition to drug standard discs on prestandardized chromatograms (TOXI-GRAMS B), additional drug standard discs are available for use with TOXI-LAB: TOXI-DISCS B-1 through B-4 and Special Standard Discs (custom formulated). These discs may be used for specific drugs in the initial drug screen, or to verify drugs detected in the initial drug screen.

To use, simply insert the standard disc of choice into the chromatogram as per the inoculation instructions, Section 5.4. The discs may be inoculated into any of the six openings in TOXI-GRAMS Blank B, or into one of the two center openings of TOXI-GRAMS B.

5.7.2 TOXI-CONTROLS
TOXI-CONTROLS Nos. 3 and 19 are positive urine controls containing various drugs detected with TOXI-LAB B. These controls and TOXI-CONTROLS Nos. 2, 4, 5, and 6 also contain drugs detected with TOXI-LAB A (analgesics, stimulants, and tranquilizers), and/or TOXI-LAB THC (cannabinoids). TOXI-CONTROL No. 1 is a negative urine control.

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TOXI-CONTROLS are designed for use as procedure controls (see Quality Control Procedure, Section 8.0). Throughout the procedure, the control should be treated the same as a urine specimen.

TOXI-CONTROLS should be used to control the TOXI-LAB procedure and materials. One procedural control per analyst per day is recommended.

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6.0 IDENTIFICATION

6.1 General Identification Procedure

6.1.1 Since most metabolites migrate to a lower position than the parent drug, observe the TOXI-GRAMS from the top downward.

6.1.2 Match position and color of any spot detected in the specimen channel with a drug standard, either included on TOXI-GRAMS B or available on other TOXI-DISCS drug standards, having the same characteristics at each stage of detection. (Specimen drug spot should be about the same size as the standard for comparison; use proper dilution, Note 10.2). **Identification is made when the specimen spot matches a standard in position and color in all stages.**

6.1.3 Metabolites and other drugs may be detected which do not match any of the TOXI-DISCS drug standards. Do not expect to identify each and every spot detected. Even an experienced analyst may be puzzled from time to time by unusual drugs and metabolites. However, TOXI-LAB includes teaching materials which will allow you to recognize many of these substances. The most important aid is the TOXI-LAB Drug Compendium which pictures the great majority of commonly detected drugs and metabolites in the PHOTO-GRAMS section in ascending R_f. In studying the PHOTO-GRAMS, you will note that many drugs have highly specific characteristics and metabolite patterns which are helpful in selecting appropriate standards for substance identification.

TOXI-LAB Special Procedures may also be used to identify unknown drugs.

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6.2 Use of the TOXI-LAB Drug Compendium

The TOXI-LAB Drug Compendium may be used to help select drug standards for the identification of unknown drug spots and/or metabolites detected in the specimen channel.

6.2.1 For each unknown drug spot, search the Compendium for drugs with similar R_f values.

6.2.2 Locate the drug and its R_f value in the Drug Index of the TOXI-LAB Drug Compendium.

6.2.3 Refer to the PHOTO-GRAMS B section of the TOXI-LAB Drug Compendium. Scan the PHOTO-GRAMS that are a full R_f above and below the selected standard spot. Compare unknown drug spot color and shape characteristics with those of the various drugs pictured in the PHOTO-GRAMS.

6.2.4 List all drugs from PHOTO-GRAMS that show similarities to the unknown drug spot in detection stages I and II.

6.2.5 Choose a drug standard disc(s), containing those drugs in question, from the additional TOXI-DISCS drug standards (see Section 5.7.1). Reanalyze the specimen comparing the specimen drug spot with TOXI-DISCS drug standard spots. **Identification is made when the specimen spot matches a standard in position and color in all stages.**

6.3 TOXI-LAB Special Procedures

TOXI-LAB Special Procedures may be used to enhance the detection of specific drugs by increasing detection specificity and/or sensitivity. See TOXI-LAB Special Procedures or the TOXI-TIPS Technical Information section of the TOXI-LAB Drug Compendium.

6.4 Identification Procedure — Discussion

The absolute position (R_f) of the standard spots may vary, within quality control guidelines, from those shown on the Detection Guide. However, the relative positions will be the same, e.g. secobarbital will always migrate above phenobarbital. Some slight variation in color, shape, and size characteristics of the standard spots may also occur from one test to another. **Therefore, it is important to recognize that the Detection Guides are for orientation purposes only, and that the unknown spots must be matched to the standard spots on the actual chromatogram.**

Two different drugs may have similar migration and staining characteristics. However, careful attention to subtle detail will frequently allow a correct interpretation.

Initially, new users should learn to differentiate artifacts and trivia from significant drug spots. Almost every chromatogram may have some artifactual material immediately above the unknown disc and at the solvent front. Common rubber stopper artifacts are depicted in the PHOTO-GRAMS B section of the TOXI-LAB Drug Compendium.

Patient information such as age, state of consciousness, drugs suspected, and drugs available can be very helpful to the analyst.

It is advisable to perform one or more tests on a urine control during initial testing to familiarize analysts with the procedure. Subsequent testing of controls may be established on a periodic basis.

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7.0 PREPARATION OF NON-URINE SPECIMENS

Urine is the single best specimen for analysis since the largest number of drugs and metabolites are found in this medium. However, other non-urine specimens may also be analyzed. Conduct the following procedures for the preparation of gastric fluid, serum or plasma, and non-biological materials (pills, powders, capsules, liquids) prior to TOXI-LAB B.

7.1 Gastric Fluid

7.1.1 Prior to analysis, filter the gastric specimen and dilute the filtrate at least 1:10.

7.1.2 Place 5 mL of dilute gastric filtrate into the extraction tube. Cap, and mix by inversion for 2 min.

7.1.3 Proceed to Section 5.2.3.

7.2 Serum or Plasma

7.2.1 Add 3 mL of water to a TOXI-TUBES B, cap, and mix for a few seconds.

7.2.2 Add 2 mL of serum or plasma to the same tube, cap, and mix by inversion for 2 min.

7.2.3 Proceed to Section 5.2.3.

7.3 Nonbiological Materials (pills, powders, capsules, liquids)

7.3.1 Crush pills to a fine powder using a clean mortar and pestle.

7.3.2 Add 2 to 3 mg of powder material or 2 to 3 µL of liquid material to a TOXI-TUBES B. Add deionized (or distilled) water to the 4.5 mL arrow. Cap and mix by inversion for 2 min.

7.3.3 Centrifuge the tube for 2-5 min.

7.3.4 Place a TOXI-DISCS Blank B into each of two concentration cups in the OMEGA-12.

7.3.5 Transfer 2 to 3 drops of the B extract to one cup and approximately 20 drops to the other cup. Save the remainder of the extract in the tube.

7.3.6 Proceed with Section 5.3.3.

NOTE: This technique will bracket most drugs except for those in extremely low concentrations. In this case the remainder of the extract may be processed or the sample size increased.

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8.0 QUALITY CONTROL PROCEDURE

The following procedure outlines acceptance/rejection criteria for variables in a TOXI-LAB B analytical run. An analytical run in this procedure is defined as all specimens run by a specific individual during a particular day.

8.1 Extraction Criteria
To control extraction, each operator should use the following procedure to demonstrate proper extraction of a urine control such as TOXI-CONTROL No. 19.

8.1.1 Analyze a urine control through the extraction process for every lot of TOXI-TUBES B used. (Extraction efficiency is consistent within a given lot of TOXI-TUBES B; Appendix II includes data used to validate this consistency.)

8.1.2 Record the lot number of TOXI-TUBES B used for each analytical run.

8.1.3 Analyze a urine control with every analytical run.

8.2 Inoculation Criteria
The inoculation process is controlled by proper disc fit. For consistent and proper placement of the disc, inspect the inoculated chromatogram and ensure that the following criteria are met.

8.2.1 The disc is in the plane of the chromatogram (i.e. lies flush with the chromatogram surface).

8.2.2 There are no spaces between the disc and chromatogram.

8.2.3 Tap the chromatogram against a firm surface. If the disc is firmly in place, it will not be dislodged.

8.3 Development Criteria
To control the migration of standards and unknowns, ensure that the following development criteria are met.

8.3.1 Secobarbital: R_f is 0.65 ± 0.05.

8.3.2 Butabarbital: R_f is 0.55 ± 0.05. The distance from the middle of the secobarbital spot to the middle of the butabarbital spot is not less than 0.5 cm ($\Delta R_f \geq 0.05$).

8.4 Detection Criteria
To control the visualization of standards and unknowns, ensure that the following detection criteria are met.

8.4.1 Stage I

8.4.1.1 Silver nitrate solution: Pale lavender spots form on a chocolate background.

8.4.1.2 Mercuric sulfate solution: Detected standards change to lavender-purple spots on a white background.

Note: Because ethinamate is a volatile compound, it may bleed from the disc into the surrounding chromatography paper and give the ethinamate spot a diffused, slightly flattened appearance. This change in spot shape is to be expected and does not indicate out-of-control conditions.

8.4.2 Stage II
UV light: Fluorescence of the diazepam spot is detected immediately and is comparable in size to other standards.

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Note: Some breakdown of the diazepam standard may be observed at approximately R_f 2.0. This is to be expected and does not indicate out-of-control conditions.

8.5 Correction Procedure

TOXI-LAB B extraction, inoculation, and concentration are easily controlled procedures that yield consistent results when properly followed. When out-of-control condition exists, correction is straightforward.

Troubleshooting is more difficult in the development and detection of chromatograms. When out-of-control conditions exist, the TOXI-LAB B procedure should be reviewed and Troubleshooting, Section 9.0, should be used as needed to make corrections.

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9.0 TROUBLESHOOTING

PROBLEMS	PROBABLE CAUSES	RECOMMENDATIONS
HIGH MIGRATION	Weak ammonium hydroxide	• Use concentrated 56% ammonium hydroxide (28% ammonia). • Buy pint bottles of ammonium hydroxide or dispense into pint containers. Pipet from working reagent vial: change daily or as needed. • Accurately measure the ammonium hydroxide required (refer to the TOXI-GRAMS jar label). • Add ammonium hydroxide to developing solution immediately prior to migration.
	Insufficient amount of ammonium hydroxide	• Add more ammonium hydroxide to the developing solution.
	Incorrect developing solvent	• Place TOXI-GRAMS into B developing solution.
	Temperature >80°F or 27°C High humidity	• Refrigerate developing solvent. • Heat-activate the chromatograms for 2 to 3 min. prior to development. • Develop the chromatogram in cool area of laboratory away from any heat source.
LOW MIGRATION	Too much ammonium hydroxide	• Decrease the volume of ammonium hydroxide in the developing solution.
	Contaminated ethyl acetate (B developing solvent)	• Check or replace B developing solvent.
	Overdevelopment	• Remove the chromatogram from developing solvent when pink dye markers are between the 9- and 10-cm line.
STREAKY MIGRATION	High humidity	• Heat-activate the chromatograms for 2 to 3 minutes prior to development.
	Improper disc fit	• Fit the disc correctly in the plane of the TOXI-GRAMS. No light should be visible surrounding it.
	High concentration of drugs	• Dilute the specimen if concentration is excessive.
SKEWED MIGRATION	Chromatogram touching sides of chromatography chamber	• Center the chromatogram in the chamber, the bottom corners of the chromatogram should not touch the side walls of the chamber.
	Heat source near chromatography chamber	• Develop the chromatogram away from the warmer or other heat source.
	Failure to mix ammonium hydroxide with developing solution	• Thoroughly mix the ammonium hydroxide with the developing fluid.
CONTAMINATION	Spattering during concentration	• Prevent spattering by beginning concentration step with a cool OMEGA-12.

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	Contaminated press card	• Dispose of cards used to press sample discs in place on TOXI-GRAMS after each use.
	Improper handling of chromatograms	• Handle the chromatogram at or above the 10-cm line to the left or right of the patient zone with clean forceps to avoid artifacts.
	Improper caps on reagent jars	• Use caps supplied with the system; avoid interchanging.
NON-VISUALIZATION Unknown Sample	Contaminated disc	• Do not contaminate sample disc with lower aqueous layer from the TOXI-TUBES.
	Excessive heat applied to disc during evaporation or after development of chromatogram	• Remove the unknown disc from the OMEGA-12 as soon as it is dry. Heat the TOXI-GRAMS minimally after development.
	Excessive delay between detection stages	• Perform detection steps with a minimal delay between stages.
	Low solvent volume in TOXI-TUBES	• Do not use TOXI-TUBES if solvent is below the arrow on the tube.
	Insufficient mixing time with TOXI-TUBES	• Mix TOXI-TUBES gently by inversion for 2 min.
	Insufficient specimen	• Refer to Sections 3.4, 5.2.1, and 7.0 for correct specimen volume and handling.
Standards	Reagent deterioration	• Make fresh reagents; use proper caps; keep reagent jars full and covered.
Blue background at Stage I detection	Moisture on chromatogram adsorbed before or after dipping in DPC (B-1 reagent)	• Maintain blue desiccant crystals in the TOXI-GRAMS jars. • After inserting unknown discs, place TOXI-GRAMS on warmer while developing solvents are being prepared. • After migration, dry chromatogram completely before dipping into the B-1 reagent (DPC). • Do not heat TOXI-GRAMS following B-1 Dip (DPC); allow to completely air dry.
	Improper cap	• Use black cork-lined cap provided with the system.
Diminished spot size (reduced sensitivity)	Dipping slowly into DPC (B-1 reagent)	• Dip quickly into and out of the B-1 reagent (DPC).
	Delay in dipping process	• Immediately transfer chromatograms from silver nitrate (B-2) to mercuric sulfate (B-3) dip.
Failure to clear chromatogram background	Deterioration of reagents with use over a period of time	• Prepare fresh reagents; refer to Section 4.0.

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10.0 TECHNICAL NOTES

10.1 To avoid dripping, hold the pipet vertically during transfer. Contamination from the lower aqueous layer may inhibit drug adsorption.

10.2 Drug spots in high concentration may be too large for correct interpretation. The following technique will allow concurrent analysis of an undiluted and a diluted specimen (approximately 1:20) and is especially recommended for suspected overdose cases.

Extract the undiluted specimen in the usual manner. For each extraction tube, place 2 TOXI-DISCS Blank B into separate concentration cups in the OMEGA-12. Concentrate 5 drops of the extract onto one of the discs and the remainder of the extract onto the second disc. Fit the dried discs into the center openings of a TOXI-GRAMS B. Develop and detect in the usual manner. If concentration is still too high, the specimen may require dilution prior to extraction.

10.3 In order to minimize loss of certain volatile drugs, the discs should be removed from the evaporation cups immediately after drying (discs will blanch when completely dry).

10.4 The second opening in the TOXI-GRAMS may be used for a second specimen, quality control, an additional TOXI-DISCS drug standard, special disc, or two different concentrations of the same specimen (see Note 10.2).

10.5 High or streaky migration of drug spots may occur if the TOXI-GRAMS are not heated (activated) for 30-60 s prior to developing.

10.6 Migration of acidic drugs is strongly influenced by the concentration of ammonium hydroxide in the developing fluid. With TOXI-GRAMS B, increasing the concentration of ammonia decreases migration. (2 to 3 mL of concentrated ammonium hydroxide should be stored in a small **tightly capped** vial and dispensed as needed. Replace with fresh ammonium hydroxide weekly or more frequently if needed.)

10.7 To prevent high migration, development should be done in a cool area of the laboratory, never in direct sunlight or close to a source of heat such as the warmer.

10.8 Excessive or prolonged heating of TOXI-GRAMS or TOXI-DISCS should be avoided as loss of heat-labile drugs such as ethinamate may occur.

10.9 The 3-mL aliquot of ammoniated developing solvent can be used for only one test. It is not necessary to wash the chromatogram chambers between each analysis, simply invert to drain.

10.10 Drug spots may appear streaky or indistinct if TOXI-GRAMS B are heated after dipping in TOXI-DIP B-1.

10.11 At Stage II, a certain degree of non-specific fluorescence may be detected from most urine specimens.

10.12 TOXI-GRAMS B may be photocopied following Stage II. Blot free of excess B-3 reagent and place in a plastic sleeve prior to photocopying.

10.13 For best results, perform the entire procedure without interruption. If it is necessary to stop, do so after any of the following steps:

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— After adding the sample to the TOXI-TUBES B and mixing.

— After centrifuging.

— After concentration of the sample extract. (Sample discs may be placed in small labeled test tubes, protected from light.)

— After the chromatogram has been developed. (Place the chromatogram in a desiccator, protected from light.)

Stopping for an extended period of time is not recommended, as increased artifacts of drug breakdown may occur.

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11.0 LIMITATIONS OF THE METHOD

Using 5 mL of urine, the threshold of detection for most drugs is approximately 1.0 µg or less per mL of urine. (Drug detection limits are included in the Drug Information Section of the TOXI-LAB Drug Compendium, however, it is recommended each laboratory establish its own detection limits.) Some substances may not be detected with TOXI-LAB, and a negative result does not mean that all drugs are absent. Questionable findings should be confirmed by employing a different methodology.

There is the possibility that other substances and/or factors not listed above may interfere with the test, for example, technical or procedural errors.

NOTE: Substitutions of other materials and/or deviations from the Instruction Manual could yield less than optimum results and as a consequence may void the warranty on this product.

11.0 Expected Values

Depending upon circumstances, the level of drug in a given specimen will vary considerably—depending upon such things as dose, route of administration, half-life, and time of sampling.

11.2 Performance Characteristics

11.2.1 Accuracy

Urine specimens with known concentrations of drugs, listed in the table below, were analyzed according to the TOXI-LAB B Drug Detection System Procedure and were shown to be positive.

11.2.2 Precision

The following drugs of abuse were evaluated. In each case a negative urine was spiked with the drug at the concentration shown. The sample was then aliquoted and 10 aliquots of each were tested with the TOXI-LAB B Drug Detection System.

SUMMARY OF DATA

Drug Name	Concentration mL	Mean R_f	$\pm R_f$	R_f Range
Phenobarbital	1.0	0.34	0.019	0.30-0.37
Secobarbital	0.5	0.69	0.011	0.67-0.70

11.2.3 Sensitivity

When using 5 mL of urine, the TOXI-LAB B Drug Detection System is sensitive to 1 µg/mL for most drugs and drug classes.

11.2.4 Specificity

A unique identification is made when a drug and/or its metabolite is detected in the unknown zone and matches an adjacent standard in position (R_f), size, shape, and color through all stages of detection.

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APPENDIX I

TOXI-GRAMS B DRUG STANDARDS

DISC	Generic Name	Brand Name	Use	Class	Reaction
B-1	Secobarbital	SECONAL®	Hypnotic	Barbiturate	Acidic
	Phenytoin	DILANTIN®	Anticonvulsant	Nonbarbiturate	Acidic
	Phenobarbital	LUMINAL®	Hypnotic	Barbiturate	Acidic
B-2	Glutethimide	DORIDEN®	Hypnotic	Nonbarbiturate	Acidic
	Pentobarbital	NEMBUTAL®	Hypnotic	Barbiturate	Acidic
	Aprobarbital	ALURATE®	Hypnotic	Barbiturate	Acidic
B-3	Ethinamate	VALMID®	Hypnotic	Nonbarbiturate	Neutral
	Amobarbital	AMYTAL®	Hypnotic	Barbiturate	Acidic
B-4	Diazepam	VALIUM®	Tranquilizer	Benzodiazepine	Neutral
	Butabarbital	BUTISOL®	Hypnotic	Barbiturate	Acidic
	Barbital	—	Hypnotic	Barbiturate	Acidic

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APPENDIX II

TOXI-LAB, Inc. TOXI-TUBES B Intra-lot Consistency Data

Intra-lot consistency is checked by the manufacturer on every batch of TOXI-TUBES B with a reference and minimum detection urine controls containing the following drugs.

Drug	Concentration of Minimum Detection Urine Control (µg/mL)	Concentration of Reference Urine Control (µg/mL)
Phenobarbital	1.0	5.0
Secobarbital	0.5	1.0

Tube lots are not approved unless sample chromatograms show all drugs present in each detection stage. For every sample tube tested, a control tube from a previously approved lot is run simultaneously.

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Toxicology Section

Section Two
Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

2.2.3 Toxi-Lab® Amine Differentiation with Acetaldehyde

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2.2.3.1 PRINCIPLE

This procedure describes a modification of the TOXI-LAB A Drug Detection System. The system is optimized for the separation and thus the differentiation of urinary phenylethylamine compounds through the addition of acetaldehyde to the TOXI-A elution solvent. The results serve to support the results of the enzyme immunoassay (EIA) screen or used in lieu of the EIA screen as described in section 2.5.2.

2.2.3.2 EQUIPMENT AND SUPPLIES

- 2.2.3.2.1 ANSYS TOXI-TUBES A (Ansys 109A-100)
- 2.2.3.2.2 TOXI-GRAMS Blank A-100 (Ansys 120A-100 or equivalent)
- 2.2.3.2.3 TOXI-DISCS Blank A-100 (Ansys 108A-100 or equivalent)
- 2.2.3.2.4 Chromatography Jar and Cap (Ansys 113A or equivalent)
- 2.2.3.2.5 Disposable Aluminum Concentration Cups (Ansys 152)
- 2.2.3.2.6 Tube Rocker (Fisher Scientific or equivalent)
- 2.2.3.2.7 Electric Warmer with Omega-12 extraction solvent concentrator (Ansys 118/153 or equivalent)
- 2.2.3.2.8 Laboratory Centrifuge

2.2.3.3 REAGENTS

- 2.2.3.1.3.1 Acetaldehyde (Acros 14951-0100 or equivalent)
- 2.2.3.1.3.2 Ammonium Hydroxide (Fisher Scientific A470-250 or equivalent)
- 2.2.3.1.3.3 Ethyl Acetate (Ansys 203 or equivalent)
- 2.2.3.1.3.4 Formaldehyde, 37% by weight (Fisher Scientific F79-500 or equivalent)
- 2.2.3.1.3.4 Sulfuric Acid (Fisher Scientific A300-500 or equivalent)

2.2.3.4 STANDARDS AND CONTROLS

- 2.2.3.4.1 Sympathomimetic amines standard disc {amphetamine, methamphetamine, phentermine, phenylpropanolamine, pseudoephedrine} (Ansys 129SA or equivalent)

2.2.3.4.2 Custom amine standard disc {MDA, MDMA, MDEA} (Ansys 129B or equivalent)

2.2.3.4.3 Toxi-Control No. 19 – {Morphine, amphetamine, imipramine, methadone, propoxyphene, phenobarbital, secobarbital and benzoylecgonine.} (Ansys 191AB).

2.2.3.4.4 Toxi-Control No. 2 – {Amphetamine, Methamphetamine, nicotine and cotinine.} (Ansys 170B).

2.2.3.4.5 Negative urine control (Ansys 170A, or equivalent).

2.2.3.5 SOLUTIONS

2.2.3.5.1 Elution Solvent

Mix 87mL ethyl acetate, 3mL methanol and 1.5mL water. Add 200μL acetaldehyde and mix well. *Store tightly capped at room temperature.*

2.2.3.6 PROCEDURE

2.2.3.6.1 Label TOXI-TUBES A for negative control, positive control (TC-19 and/or TC-2) and for case samples with appropriate laboratory numbers.

2.2.3.6.2 Transfer 5mL each of urine specimen, negative urine and positive control to TOXI-TUBE A. Place on rocker for ≥10 minutes.

2.2.3.6.3 Centrifuge tube at 2500 rpm for ≥10 minutes.

2.2.3.6.4 Place appropriate number of concentration cup into Omega-12 extraction solvent concentrator. To each cup add a Toxi-A disc. Allow cup to warm prior to the addition of extract.

2.2.3.6.5 Transfer upper solvent layer from tube into pre-heated concentration cup in Omega-12 extraction solvent concentrator.

2.2.3.6.6 Evaporate solvent on disc on electric warmer. Take care not to over dry disc.

2.2.3.6.7 Insert sympathomimetic amines standard disc and/or custom amine disc into labeled channel on 6-channel TOXI-GRAM A.

2.2.3.6.8 Place specimen disc into labeled channel on 6-channel TOXI-GRAM A.

2.2.3.6.9 Heat the GRAM, with the disc end slightly off the warmer edge.

2.2.3.6.10 Add 3mL of developing solution to chromatography jar.

2.2.3.6.11 To developing solution, add the volume of ammonium hydroxide indicated on TOXI-GRAM A box and swirl vigorously.

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2.2.3.6.12 Place GRAM into chromatography jar. Allow dye marker to migrate to ≅ 10 cm.

2.2.3.6.13 Remove GRAM from jar and place face down on warmer for 30-60 seconds.

2.2.3.6.14 Place GRAM into TOXI-DIP A-1 for formaldehyde fuming for ≥5 minutes.

2.2.3.6.15 Slowly dip GRAM into TOXI-DIP A-2 [concentrated sulfuric acid]. Remove and observe the R_f value and color characteristics of compounds versus those exhibited by the compounds in the standard disc for 15 to 60 seconds.

2.2.3.6.16 Place GRAM into a page protector and label samples and controls.

2.2.3.6.17 Photocopy GRAM, with header information, for each case file.

2.2.3.7 DETECTION AND IDENTIFICATION CRITERIA

2.2.3.7.1 The phenylethylamine constituents in the standard disc should exhibit the elution order and color characteristics indicated in the chart below.

2.2.3.7.2 Positive control should establish the presence of appropriate phenylethylamine compounds by exhibiting the proper R_f and color characteristics.

2.2.3.7.3 Negative control should not exhibit characteristics supporting the presence of phenylethylamine compounds or contain interfering substances.

2.2.3.7.4 The method supports the presence of a phenylethylamine class drug compound if there are no significant differences in the R_f value and color characteristics for the sample versus appropriate standard. Consideration should be given to concentration differences and/or interfering/coeluting substances.

2.2.3.7.5 The following table indicates the elution order and color characteristics of commonly encountered phenylethylamines and interfering substances. Absolute R_f is provided only to establish elution order.

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Compound	R_f	Stage I Color Characteristics
Ephedrine/pseudoephedrine	0.85	Yellow → green center
Phenylpropanolamine (PPA)	0.70	Yellow → green center
Amphetamine	0.55	Yellow → brown center
3,4-Methylenedioxyamphetamine (MDA)	0.55	Deep purple-blue
β-Phenylethylamine	0.52	Yellow → brown center
Para-Methoxyamphetamine (PMA)	0.50	Deep purple-blue
Labetalol	0.50	Melon yellow
Phentermine	0.39	Yellow → brown center
3,4-Methylenedioxyethylamphetamine (MDE)	0.35	Deep purple-blue
Methamphetamine	0.25	Yellow → brown center
3,4-Methylenedioxymethamphetamine (MDMA)	0.25	Deep purple-blue
Normeperidine	0.18	Yellow → green center

2.2.3.8 REFERENCES

2.2.3.8.1 TOXI-LAB Drug Compendium, Adams, D.J., ed., ANSYS Diagnostics, Inc., 1998.

2.2.3.8.2 Moore, K., *Amphetamines/Sympathomimetic Amines*. pp. 277, *in:* Principles of Forensic Toxicology. Levine, B. ed., AACC, 1999.

2.2.3.8.3 Phenylethylamines, ANSYS Diagnostics, Inc., 2000.

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Toxicology Section

Section Two
Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

2.2.3 Toxi-Lab® Amine Differentiation with Acetaldehyde

Revision #	Issue Date	History
0	10-18-02	SOP format

Approval

Technical Leader: ____________________ **Date:** __________
S C Williamson

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QC Manager: ____________________ **Date:** __________
Rick D. Groff

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Toxicology Section

Section Two
Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

2.2.4 Toxi-Lab® THC II-PLUS 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (Δ9-THC-COOH) Detection System

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2.2.4.2 SUMMARY PROCEDURE

2.4.4.2.1 STANDARDS

2.4.4.2.1.1 Stock Standard Solution
100μg/mL (+) 11-nor-9-carboxy-Δ^9-THC (Radian T-006 or equivalent).

2.4.4.2.1.2 Working Standard Solution (1800ng/mL)
Add 900μL Stock Solution to 49.1mL Methanol. Solution is stable for six months when stored at 4°C.

2.4.4.2.2 CONTROLS

2.4.4.2.2.1 60ng/mL Carboxy-THC Positive Control Urine
Add 200μL of working standard solution to 6mL of negative urine and mix.

2.4.4.2.2.2 Negative Control Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.4.4.2.3 PROCEDURE

2.4.4.2.3.1 Initial set-up
Label sample tubes and extraction cartridges for the negative control (NC), positive control (PC), and appropriate laboratory numbers without prefix.

2.4.4.2.3.2 Sample Preparation
Transfer 6 mL of urine specimen, negative control urine, and positive control urine to test tube.

2.4.4.2.3.3 Sample Hydrolysis

- To 6mL of urine, add 12 drops 11.8N KOH.
- Vortex
- Allow to hydrolyze for 10 minutes.
- Add 1.5mL glacial acid.
- Vortex

2.4.4.2.3.4 Extraction

- Condition cartridge with 1mL methanol. Aspirate at approximately 5 in. Hg. ***Do not allow the disc to dry.***
- Add acidified samples to cartridge reservoirs. Aspirate such that the sample passes through the column no faster than 2mL/min.
- Once the sample is completely through the reservoir, remove filter.
- Add 1mL 20% acetic acid.
- Aspirate ≥2 minutes at 10-12 in. Hg.
- Add 500mL wash reagent.
- Aspirate at 10-12 in. Hg.
- After solvent has past through, allow to aspirate ≥2 minutes.
- Remove disc from cartridge and place into a pre-heated concentrated cup to remove all residual moisture.

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2.4.4.2.3.5 TLC

- Place disc into labeled three or 10-channel TOXI-GRAM for THC-II.
- Add THC-COOH standard disc.
- Heat the GRAM, with the disc end slightly off the warmer edge, for 30-60 seconds.
- Add 12.5mL of developing solution to chromatography tank (10-channel) or 3mL solution to chromatography jar (3-channel).
- Place gram into chromatography tank or jar. Allow dye marker to migrate to ≅ 4 cm. [***This only takes 2-3 minutes***]
- Remove GRAM from tank/jar and place face down on warmer for 1-2 minutes.

- Dip GRAM into TOXI-DIP 1, hold to dry until GRAM becomes speckled.
- Place GRAM into TOXI-DIP THC-2 until scarlet spots develop.
- Place GRAM in hood so that the diethylamine (DEA) can evaporate. If any DEA is present when the HCl is added, fuming will occur.
- Place GRAM on to a page protector. With bulb pipet, add concentrated HCl to just cover GRAM. Note desired color change to deep purple.
- Close page protector and label samples and controls.
- Photocopy GRAM, with header information, for each casefile.

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Toxicology Section

Section Two
Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

Revision #	Issue Date	History
0	10/91	Original Issue
1	11-27-01	Introduction into Reformatted SOP Manual
2	04-25-02	THC-II Method Summary Added
3	10-18-02	Refinements, additional methods added to alternative method binder

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.1 Amphetamine and Methamphetamine Extraction Employing the Ansys® Diagnostics Spec·Plus™ DAU Column

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2.3.1.1 BACKGROUND

Amphetamine and methamphetamine are sympathomimetic drugs that mimic the actions of naturally occurring stimulatory neurotransmitters. Although still prescribed for the treatment of attention deficit disorder (ADD), narcolepsy, and obesity, these compounds have a high potential for abuse. Methamphetamine is produced clandestinely often through the reduction of ephedrine/pseudoephedrine. Psychological side effects may include agitation, nervousness, restlessness, and paranoia. Physiological effects may include mydriasis, insomnia, increased blood pressure and heart rate. The manifestation of adverse affects is dependent on the time since drug administration.

2.3.1.2 PRINCIPLE

This procedure outlines the use of the ANSYS® Diagnostics, Inc SPEC·PLUS™ 3ml DAU column for the extraction of amphetamine and methamphetamine and amine compounds, from urine. ANSYS Technologies' SPEC™ Solid Phase Extraction products are manufactured with polypropylene plastic and bonded-silica impregnated on a glass fiber disc. The DAU column utilizes a copolymer sorbent which combines a strong cation exchange phase with a non-polar phase (reversed phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the urine sample. The copolymer binds the analyte primarily with ionic interactions with the anionic sorbent and to a lesser extent, by hydrophobic interactions. The cation exchange component (anionic sorbent) of the phase is effective for recovering amines which are present in the urine sample in a cationic form.

For the extraction of amphetamine, methamphetamine and other phenethylamines of interest, the urine is adjusted with a phosphate buffer and applied to a pre-conditioned SPE column. This pH adjustment maximizes the ionic character of the analyte and the sorbent to take full advantage of the cation exchange mechanism. The conditioning creates an environment which allows for optimal interaction between the sorbent and

the analytes of interest. The column is subsequently washed with an aqueous solvent, to selectively remove matrix components and interfering substances from the column. Next, the column is to dried to remove traces of solvent. When the column is dry, the analytes of interest are recovered from the column by disrupting the ionic bonds with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.1.3 EQUIPMENT AND SUPPLIES

2.3.1.3.1 SPEC·PLUS™ 3ml DAU column (Ansys 532-DAU)

2.3.1.3.2 Drybath (Fisher or equivalent)

2.3.1.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.1.3.4 Vacuum Manifold/pump

2.3.1.3.5 Glassware
16X100 Test Tubes (Fisher 14-961-29 or equivalent)
16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)
Snap Caps (Fisher 05-538-41N or equivalent)
GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.1.3.6 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

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2.3.1.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.1.4.1 1.0 M Potassium hydroxide

2.3.1.4.2 0.1M Phosphate Buffer, pH 6.0

2.3.1.4.3 0.1M Acetic acid

2.3.1.4.4 1% Acidic Methanol

2.3.1.4.5 Isooctane (Fisher O-299-1 or equivalent)

2.3.1.4.6 Methanol (Fisher A412-4 or equivalent)

2.3.1.4.7 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.1.4.8 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.1.4.9 Elution Solvent
80ml ethyl acetate, 20 ml methanol, 2ml of NH_4OH
Prepare fresh.

2.3.1.4.10 1M Potassium phosphate dibasic (K_2HPO_4)

2.3.1.4.11 Derivatizing Agents - Select from the following:

Heptafluorobutyric Acid Anhydride (HFAA) (Pierce 63164 or equivalent)
Pentafluoropropionic Acid Anhydride (PFAA) (Pierce 65193 or equivalent)

2.3.1.5 QUALITATIVE NON-EXTRACTED STANDARDS (NES)

2.3.1.5.1 Sources of Standards

Standard (1.0mg/mL)	Potential Vendors
Methamphetamine	Cerilliant M-009, Alltech 010013
Amphetamine	Cerilliant A-007, Alltech 010023
MDMA	Cerilliant M-013, Alltech 014093
MDA	Cerilliant M-012, Alltech 014603
Phenylpropanolamine	Cerilliant P-038, Alltech 6017803
Phentermine	Cerilliant P-023, Alltech 017833
Ephedrine	Cerilliant E-024, Alltech 017403
Pseudoephedrine	Cerilliant P-035, Alltech 6013213
PMA	Cerilliant P-050

2.3.1.5.2 Non-extracted Derivatized Standards

2.3.1.5.2.1 Prepare a minimum of methamphetamine, amphetamine, and ephedrine anhydride derivatives. Additional standards should be prepared as necessary indicated by current drug therapy and/or examination of GC/MSD data.

2.3.1.5.2.2 Add 10μL of stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.1.7.2.

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2.3.1.6 QUALITATIVE CONTROLS

2.3.1.6.1 Extracted Positive Control

Toxi-Control No. 2, UTAK 98814, or equivalent control which contains both Amphetamine and Methamphetamine in the appropriate concentrations.

2.3.1.6.2 Extracted Negative Control

Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.3.1.7 PROCEDURE

2.3.1.7.1 Initial set-up

Label the test tubes and GC/MS vials with microinserts.

- Negative Control
- Positive Control
- Appropriate Laboratory Numbers

2.3.1.7.2 Manual Extraction Procedure Utilizing the SPEC·PLUS™ •3ml DAU column

2.3.1.7.2.1 Transfer 1mL of urine specimen, negative urine or appropriate control to the properly labeled test tube.

2.3.1.7.2.2 Add 500µL of 0.1M phosphate buffer, pH 6.0, and vortex.

2.3.1.7.2.3 Insert labeled SPEC·PLUS™ 3mL DAU column into vacuum manifold.

2.3.1.7.2.4 Add 200µL of methanol to column and aspirate at approximately 5 in. Hg (17 kPa) for approximately 1 minute.

2.3.1.7.2.5 Pour prepared sample into column and aspirate at approximately 5 in. Hg (17 kPa).

2.3.1.7.2.6 Add 500µL of 0.1M acetic acid and aspirate at approximately 5 in. Hg (17 kPa).

2.3.1.7.2.7 Increase vacuum to 10-20 in. Hg (34-68 kPa) and dry the extraction disc for a minimum of 1 minute.

2.3.1.7.2.8 Add 500µL of Methanol to the column and aspirate at approximately 5 in. Hg (17 kPa).

2.3.1.7.2.9 Increase the vacuum to 10-20 in Hg (34-68 kPa) and dry the disc for a minimum of 1 minute.

2.3.1.7.2.10 Open vacuum manifold, wipe collection tips, and insert rack containing the 16X144mm tapered tip centrifuge tubes.

2.3.1.7.2.11 Add 800µL of elution solvent to column and aspirate slowly, < 3 in. Hg (10kPa).

2.3.1.7.2.12 Increase vacuum to 5 in. Hg (17 kPa) to assist final amount of elution solvent through the disc.

2.3.1.7.2.13 Remove the tapered tip centrifuge tubes containing the collected samples from rack.

2.3.1.7.2.14 Add 50µL of 1% acidic methanol and vortex.

2.3.1.7.2.15 Evaporate to dryness under a gentle stream of nitrogen at approximately 35°C.

2.3.1.7.2.16 In the hood add 50µL of HFAA or PFAA, cap, and vortex.

2.3.1.7.2.17 Heat for 20 minutes at 70°C.

2.3.1.7.2.18 Cool to room temperature.

2.3.1.7.2.19 Add 1 mL of Isooctane and 1mL of 1M K_2HPO_4.

2.3.1.7.2.20 Cap and vortex.

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2.3.1.7.2.21 Incubate at ~60°C for 15 minutes.
2.3.1.7.2.22 Cool.
2.3.1.7.2.23 Vortex
2.3.1.7.2.24 Centrifuge at 100rpm for 5 minutes to separate the layers
2.3.1.7.2.25 Transfer the isooctane (top) layer to an appropriately labeled ALS vial.

2.3.1.7.3 Automated Extraction Procedure Utilizing SPEC·PLUS™ - 3ml DAU column.

2.3.1.7.3.1 Refer to the following attached methods/printouts.

2.3.1.7.4 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

2.3.1.7.4.1 Inject 1 μL into GC/MS using the ALS.

2.3.1.7.4.2 Analyze sample extract in full scan acquisition. Refer to current GC/MSD method printout for current analysis parameters.

2.3.1.7.5 Detection and Identification Criteria

2.3.1.7.5.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus that of an authenticated standard.
2.3.1.7.5.2 Acceptable retention time window is ±5%.

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2.3.1.8 REFERENCES

2.3.1.8.1 Automated SPEC® · Solid Phase Extraction Protocols for Drugs of Abuse Using the RapidTrace™ SPE Workstation, ANSYS Diagnostics, 1997.
2.3.1.8.2 SPEC·PLUS™·3ML·DAU Drugs of Abuse in Urine Extraction Applications, ANSYS Diagnostics, 1999.
2.3.1.8.3 Instructions for Urine of SPEC·Solid Phase Extraction Columns, SPEC·PLUS™ Solid Phase Extraction Columns with Filter, ANSYS Diagnostics, 1997.
2.3.1.8.4 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

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Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.1 Amphetamine and Methamphetamine Extraction Employing the Ansys® Diagnostics Spec·Plus™ DAU Column

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-17-02	Refinements

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Approval

Technical Leader: ______________________ **Date:** __________
S. C. Williamson

Issuance

QC Manager: ______________________ **Date:** __________
Rick D. Groff

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Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.2 Extraction of Amphetamines Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

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2.3.2.1 BACKGROUND

Amphetamine and methamphetamine are sympathomimetic drugs that mimic the actions of naturally occurring stimulatory neurotransmitters. Although still prescribed for the treatment of attention deficit disorder (ADD), narcolepsy, and obesity, these compounds have a high potential for abuse. Methamphetamine is produced clandestinely often through the reduction of ephedrine/pseudoephedrine. Psychological side effects may include agitation, nervousness, restlessness, and paranoia. Physiological effects may include mydriasis, insomnia, increased blood pressure and heart rate. The manifestation of adverse affects is dependent on the time since drug administration.

2.3.2.2 PRINCIPLE

This procedure outlines the use of the UCT 200mg CLEAN SCREEN® DAU column for the extraction of Amphetamines from urine. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the urine sample.

The cation exchanger will allow the anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] The cation exchanger component of the phase is effective for amines which are present in the urine sample in a cationic form bonding ionically to the sorbent.[2]

For the extraction of amphetamines; the urine is adjusted to pH 6 with a phosphate buffer to maximize the ionic character of the analyte, and loaded onto a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the amine functional groups present on the drug and the anionic sulfonic acid

exchanger on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.2.3 EQUIPMENT AND SUPPLIES

2.3.2.3.1 200 mg CLEAN SCREEN® extraction column (ZSDAU020 or ZCDAU020 or equivalent)

2.3.2.3.2 Drybath (Fisher or equivalent)

2.3.2.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.2.3.4 Vacuum Manifold/pump

2.3.2.3.5 pH paper (Fisher 09-876-17 or equivalent)

2.3.2.3.6 Glassware

16X100 Test Tubes (Fisher 14-961-29 or equivalent)

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.2.3.6 Gas Chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

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2.3.2.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.2.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.2.4.2 Methylene Chloride (Fisher D37-4 or equivalent)

2.3.2.4.3 Dimethylformamide (DMF) (Fisher D119-500 or equivalent

2.3.2.4.4 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.2.4.5 Isopropanol (Fisher A416-1 or equivalent)

2.3.2.4.6 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.2.4.7 100mM Phosphate Buffer (pH 6.0)
2.3.2.4.8 100mM Acetic Acid
2.3.2.4.9 Elution Solvent
Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***
2.3.2.4.10 Derivatizing Agents - Select from the following:
Heptafluorobutyric Acid Anhydride (HFAA) (Pierce 63164 or equivalent)
Pentafluoropropionic Acid Anhydride (PFAA) (Pierce 65193 or equivalent)

2.3.2.5 QUALITATIVE NON-EXTRACTED STANDARDS (NES)

2.3.2.5.1 Sources of Standards

Standard (1.0mg/mL)	Potential Vendors
Methamphetamine	Cerilliant M-009, Alltech 010013
Amphetamine	Cerilliant A-007, Alltech 010023
MDMA	Cerilliant M-013, Alltech 014093
MDA	Cerilliant M-012, Alltech 014603
Phenylpropanolamine	Cerilliant P-038, Alltech 6017803
Phentermine	Cerilliant P-023, Alltech 017833
Ephedrine	Cerilliant E-024, Alltech 017403
Pseudoephedrine	Cerilliant P-035, Alltech 6013213
PMA	Cerilliant P-050

2.3.2.5.2 Non-extracted Derivatized Standards
2.3.2.5.2.1 Prepare a minimum of methamphetamine, amphetamine, and ephedrine anhydride derivatives. Additional standards should be prepared as necessary indicated by current drug therapy and/or examination of GC/MSD data.
2.3.2.5.2.2 Add 10µL of stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.2.7.2.

2.3.2.6 QUALITATIVE CONTROLS

2.3.2.6.1 Extracted Positive Control
Toxi-Control No. 2, UTAK 98814, or equivalent control which contains both Amphetamine and Methamphetamine in the appropriate concentrations.
2.3.2.6.2 Extracted Negative Control
Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

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2.3.2.7 PROCEDURE

2.3.2.7.1 Initial set-up

Label test tubes and GC/MSD vials with microinserts.

- Negative Control
- Positive Control
- Appropriate Laboratory Numbers

2.3.2.7.2 Extraction Procedure Utilizing the 200 mg CLEAN SCREEN® Extraction Column

2.3.2.7.2.1 Transfer 5mL of urine specimen, negative urine or appropriate Toxi-Control to the appropriate labeled test tube.

2.3.2.7.2.2 Add 2mL 100mM phosphate buffer and vortex. Verify that pH is 6.0 ± 0.5, adjust pH with 100mM monobasic or dibasic sodium phosphate, as necessary.

2.3.2.7.2.3 Insert labeled CLEAN SCREEN® extraction column into vacuum manifold.

2.3.2.7.2.4 Add 3mL of methanol to column and aspirate at ≤3 in. Hg.

2.3.2.7.2.5 Add 3mL of DI H_2O to column and aspirate at ≤3 in. Hg.

2.3.2.7.2.6 Add 1mL of 100mM phosphate buffer (pH 6.0) to column and aspirate at ≤3 in. Hg.

2.3.2.7.2.7 Pour sample onto column and aspirate at ≤3 in. Hg.

2.3.2.7.2.8 Wash column with 3mL DI H_2O and aspirate at ≤3 in. Hg.

2.3.2.7.2.9 Wash column with 1mL 100mM acetic acid and aspirate at ≤3 in. Hg.

2.3.2.7.2.10 Wash column with 3mL methanol and aspirate at ≤3 in. Hg.

2.3.2.7.2.11 Dry column for ≥ 5minutes at ≥10 inches Hg.

2.3.2.7.2.12 Open vacuum manifold, wipe collection tips, and insert rack containing labeled 16X144mm tapered tip centrifuge tubes.

2.3.2.7.2.13 Add 3mL of elution solvent to column and aspirate slowly, < 3 in. Hg.

2.3.2.7.2.14 Add 30µL of DMF to eluate.

2.3.2.7.2.15 Evaporate eluate to ~30µL at ≤40°C under a gentle stream of nitrogen.

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2.3.2.7.2.16 Add 50μL of PFFA or HFFA, cap, and vortex.

2.3.2.7.2.17 Heat for 20 minutes at 70°C.

2.3.2.7.2.18 Evaporate to dryness at ≤40°C.

2.3.2.7.2.19 Reconstitute with 100μL ethyl acetate.

2.3.2.7.2.20 Transfer to the appropriately labeled ALS vial.

2.3.2.7.3 Automated Extraction Procedure Utilizing 200 mg CLEAN SCREEN® Extraction Column

2.3.2.7.3.1 Refer to the following attached methods/printouts.

2.3.2.7.4 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

2.3.2.7.4.1 Inject 1 μL into GC/MS using the ALS.

2.3.2.7.4.2 Analyze sample extract in full scan acquisition. Refer to attached GC/MSD method printout for current analysis parameters.

2.3.2.7.5 Detection and Identification Criteria

2.3.2.7.5.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus that of an authenticated standard.

2.3.2.7.5.2 Acceptable retention time window is ±5%.

2.3.2.8 REFERENCES

2.3.2.8.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

2.3.1.8.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.2 Extraction of Amphetamines Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-17-02	Refinements

Approval

Technical Leader: ____________________ **Date:** __________
S. C. Williamson

Issuance

QC Manager: ____________________ **Date:** __________
Rick D. Groff

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Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.3 Extraction of Benzodiazepines Employing the Ansys® Diagnostics Spec·Plus™ DAU Column

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2.3.3.1 BACKGROUND

Refer to manual section 2.4.3.

2.3.3.2 PRINCIPLE

This procedure outlines the use of the ANSYS® Diagnostics, Inc SPEC·PLUS™ 3ml SPE column for the extraction of benzodiazepines from urine. ANSYS Technologies' SPEC™ Solid Phase Extraction products are manufactured with polypropylene plastic and bonded-silica impregnated on a glass fiber disc. For benzodiazepines, a non-polar phase (reversed phase) retention mechanism is utilized. to interact effectively, with analytes of interest and minimally with interfering substances in the urine sample. The non-polar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[4]

Benzodiazepines form glucuronide conjugates to facilitate their excretion. An enzymatic hydrolysis is required to free them from the glucuronide sugar moiety. For the extraction of benzodiazepines, the urine is adjusted to pH 10.8 with a phosphate buffer, to maximize the hydrophobic/non-polar character of the analyte and the sorbent, and applied to a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with an aqueous solvent, to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of solvent. When the column is dry, the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.3.3 EQUIPMENT AND SUPPLIES

2.3.3.3.1 SPEC·PLUS™ - 3mL DAU SPE column (Ansys 532-DAU or equivalent)

2.3.3.3.2 Drybath (Fisher or equivalent)

2.3.3.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.3.3.4 Vacuum Manifold/pump

2.3.3.3.5 Glassware

16X100 Test Tubes (Fisher 14-961-29 or equivalent)

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

pH paper (Fisher 09-876-17 or equivalent)

2.3.3.3.6 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

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2.3.3.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.3.4.1 Isooctane (2,2,4 – trimethylpentane) (Fisher O-299-1 or equivalent)

2.3.3.4.2 1.0M Acetate Buffer, pH 3.8

2.3.3.4.3 1.5 M Phosphate Buffer, pH 10.8

2.3.3.4.4 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.3.4.5 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.3.4.6 Elution Solvent

To 98mL ethyl acetate add 2ml NH_4OH, mix.

Make Fresh.

2.3.3.4.7 Deionized/distilled water

2.3.3.4.8 β-Glucuronidase (Patella vulgata) Options

- Prepare from Patella vulgata Type L-II powder (Sigma G8132 or equivalent)
- Prepared Helix pomatia Type H-2 Solution (Sigma G0876 or equivalent)

2.3.3.4.9 Silylation Reagent Options

- MTBSTFA / 1% t-BDMCS (Pierce 48925 or equivalent)

2.3.3.5 QUALITATIVE STANDARDS AND CONTROLS

2.3.3.5.1 Sources of Standards

Standards in Solution	***Potential Vendors***
Alprazolam	Cerilliant A-903, Alltech 01427
α-Hydroxyalprazolam	Cerilliant A-907, Alltech 01545
Bromazepam	Cerilliant B-903, Alltech 6013563
Chlordiazepoxide	Cerilliant C-022
Norchlordiazepoxide	Alltech 6013433
Clonazepam	Cerilliant C-907, Alltech 017943
7-Aminoclonazepam	Cerilliant A-916
Desalkylflurazepam	Cerilliant D-915
Diazepam	Cerilliant D-907, Alltech 017213
Estazolam	Cerilliant E-901, Alltech 601560
Flurazepam	Cerilliant F-003, Alltech 017953
Flunitrazepam	Cerilliant F-907, Alltech 6015123
7-aminoflunitrazepam	Cerilliant A-911
2-Hydroxyethylflurazepam	Cerilliant F-902
Lorazepam	Cerilliant L-901, Alltech 013583
Medazepam	Alltech 013573
Midazolam	Cerilliant M-908
4-hydroxymidazolam	Cerilliant H-902
Nitrazepam	Cerilliant N-906, Alltech 017933
Nordiazepam	Cerilliant N-905, Alltech 013453
Oxazepam	Cerilliant O-902, Alltech 013703
Temazepam	Cerilliant T-907, Alltech 013833
Triazolam	Cerilliant T-910, Alltech 014283
α-Hydroxytriazolam	Cerilliant T-911

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2.3.3.5.2 Non-extracted Standards

2.3.4.5.2.1 Run necessary NES analytical standards as indicated by examination of GC/MSD data.

2.3.4.5.2.2 Dilute 1.0mg/mL standard solution to 250ug/mL with methanol.

2.3.3.5.3 Non-extracted Derivatized Standards

2.3.3.5.3.1 Prepare a minimum of Oxazepam, Temazepam, Nordiazepam and Lorazepam TMS standards. Additional standards should be prepared as necessary indicated by current drug therapy or examination of GC/MSD data.

2.3.3.5.3.2 Add 10µL of stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.3.6.2.4.

2.3.3.5.4 Extracted Positive Controls

2.3.3.5.4.1 Liquid Urine Control containing a minimum of Oxazepam or Nordiazepam (BioRad 443, Utak 88121 or equivalent)

2.3.3.5.4.2 Drug Mix Control

Drug Mix: Drug Mix (Alltech 601826 {Medazepam, Oxazepam, Lorazepam, Diazepam, Temazepam, and Bromazepam} or similar)
Negative Urine: Ansys 170A, Utak 88121-CDF (L) or equivalent.
Preparation: Pipette 10µL of drug mixture into 1mL of negative urine.

2.3.3.5.4.3 Enzyme Controls

Conjugated Standard: Oxazepam Glucuronide (Alltech 01541 or equivalent).
Negative Urine: Ansys 170A, Utak 88121-CDF (L) or equivalent.
Preparation: Pipette 10µL of conjugated standard into 1mL of negative urine. Prepare two controls to allow for both the presence and absence of glucuronidase.

2.3.3.5.5 Extracted Negative Control

Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

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2.3.3.6 PROCEDURE

2.3.3.6.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, tapered-bottom derivatization tubes and GC/MS vials with microinserts as follows for the negative control, positive controls, Oxazepam Glucuronide controls (with and without glucuronidase) and appropriate laboratory numbers without prefix. Label tapered-bottom derivatization tubes and GC/MS vials with microinserts for NES derivatized standards.

2.3.3.6.2 Manual Extraction Procedure Utilizing the SPEC·PLUS™ •3ml DAU column

2.3.3.6.2.1 Sample Preparation

Transfer 1.0mL of urine specimen, negative urine or positive control to labeled extraction test tube.

2.3.3.6.2.2 Sample Hydrolysis

For each extraction tube:

- Add 200µL 1.0M acetate buffer, pH 3.8

- Vortex.
- Verify that the resulting pH is approximately 4.
- If necessary adjust pH.

- Add 200μL β-Glucuronidase.
  - Cap and vortex *gently* to mix.
- Place in 37°C laboratory oven or waterbath for three hours.
- Allow samples to cool before proceeding with solvent extraction.

2.3.3.6.2.3 Extraction

For each extraction tube:

- Add 1.5mL of phosphate buffer (pH 10.8)
  - Vortex.
  - Resulting pH should be approximately 10.
  - If necessary, adjust pH.
- Centrifuge at 3500 rpm for ≥5 minutes.
- Insert labeled SPE·PLUS™·3ml·DAU column in the vacuum manifold.
- Add 200μL of methanol to the column. Wait for 1 minute.
- Decant sample into column and aspirate at approximately 3-5 in. Hg (10-17kPa)
- Add 1mL of deionized water to column and aspirate at approximately 3-5 in. Hg (10-17kPa)
- Increase vacuum to 10-20 in. Hg (34-68kPa) and dry extraction disc for approximately 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 800μL of elution solvent and apply ***gentle*** vacuum of <3 in. Hg (10 kPa) to aspirate the sample into the collection tube
- Increase vacuum to approximately 5 in. Hg (17 kPa) to assist the final amount of elution solvent through the disc.

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- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at 60°C.

2.3.3.6.2.4 Derivatization

- In hood, add 100μL silylating agent.
- Cap tubes with snap caps.
- Vortex.
- Heat tube in 90°C dry bath for 30 minutes.
- Remove from dry bath and allow to cool.
- Add 100μL of isooctane.
- Vortex.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

2.3.3.6.3 Automated Extraction Procedure Utilizing SPEC·PLUS™ - 3ml DAU column.

2.3.1.6.3.1 Refer to the current methods/printouts.

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2.3.3.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

2.3.3.7.1 Analysis Parameters

2.3.3.7.1.1 Inject 1 μL into GC/MSD using the ALS.

2.3.3.7.1.2 Analyze sample extract in full scan acquisition.

2.3.3.7.1.3 Refer to GC/MSD method printout for current analysis parameters.

2.3.3.7.2 Detection and Identification Criteria

2.3.3.7.2.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

- Acceptable retention time window is +/- 5%.

2.3.3.8 REFERENCES

2.3.1.8.1 Automated SPEC® · Solid Phase Extraction Protocols for Drugs of Abuse Using the RapidTrace™ SPE Workstation, ANSYS Diagnostics, 1997.

2.3.1.8.2 SPEC·PLUS™·3ML·DAU Drugs of Abuse in Urine Extraction Applications, ANSYS Diagnostics, 1999.

2.3.1.8.3 Instructions for Urine of SPEC·Solid Phase Extraction Columns, SPEC·PLUS™ Solid Phase Extraction Columns with Filter, ANSYS Diagnostics, 1997.

2.3.1.8.4 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation
2.3.3 Extraction of Benzodiazepines Employing the Ansys® Diagnostics Spec·Plus™ DAU Column

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-18-02	Refinements

Approval

Technical Leader: ______________________ **Date:** __________
S. C. Williamson

Issuance

QC Manager: ______________________ **Date:** __________
Rick D. Groff

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Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.4 Extraction of Benzodiazepines Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

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2.3.4.1 BACKGROUND

Refer to manual section 2.4.3.

2.3.4.2 PRINCIPLE

This procedure outlines the use of the 200mg UCT CLEAN SCREEN® DAU Column for the extraction of Benzodiazepines from urine The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the urine sample.

The retention mechanisms for the benzodiazepines are hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] Benzodiazepines form glucuronide conjugates to facilitate their excretion. An enzymatic hydrolysis is therefore required to free them from the glucuronide sugar moiety. For the extraction of benzodiazepines, the hydrolyzed urine is loaded onto a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by hydrophobic interaction of the functional groups present on both the analyte and the sorbent. The column is subsequently washed to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry, the analytes of interest are recovered from the column with a basic organic solvent. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.4.3 EQUIPMENT AND SUPPLIES

2.3.4.3.1 200 mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

2.3.4.3.2 Drybath (Fisher or equivalent)

2.3.4.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.4.3.4 Vacuum Manifold/pump

2.3.4.3.5 Glassware

16X100 Test Tubes (Fisher 14-961-29 or equivalent)

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.4.3.6 pH paper (Fisher 09-876-17 or equivalent)

2.3.4.3.7 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

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2.3.4.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.4.4.1 ethanol (Fisher A412-4 or equivalent)

2.3.4.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.4.4.3 Hexane (Fisher H-292-1 or equivalent)

2.3.4.4.4 Deionized/distilled water

2.3.4.4.5 100 mM Phosphate buffer, pH 6.0

2.3.4.4.6 20 % Acetonitrile in 0.1 M phosphate buffer, pH 6.0

2.3.4.4.7 β-Glucuronidase (Patella vulgata)

2.3.4.4.8 Silylation Reagent Options

- BSTFA + 1% TMCS (Pierce 38831 or equivalent)

2.3.4.5 QUALITATIVE STANDARDS AND CONTROLS

2.3.4.5.1 Sources of Standards

Standards in Solution	*Potential Vendors*
Alprazolam	Cerilliant A-903, Alltech 01427
α-Hydroxyalprazolam	Cerilliant A-907, Alltech 01545
Bromazepam	Cerilliant B-903, Alltech 6013563
Chlordiazepoxide	Cerilliant C-022
Norchlordiazepoxide	Alltech 6013433
Clonazepam	Cerilliant C-907, Alltech 017943
7-Aminoclonazepam	Cerilliant A-916
Desalkylflurazepam	Cerilliant D-915
Diazepam	Cerilliant D-907, Alltech 017213

Standards in Solution	*Potential Vendors*
Estazolam	Cerilliant E-901, Alltech 601560
Flurazepam	Cerilliant F-003, Alltech 017953
Flunitrazepam	Cerilliant F-907, Alltech 6015123
7-aminoflunitrazepam	Cerilliant A-911
2-Hydroxyethylflurazepam	Cerilliant F-902
Lorazepam	Cerilliant L-901, Alltech 013583
Medazepam	Alltech 013573
Midazolam	Cerilliant M-908
4-hydroxymidazolam	Cerilliant H-902
Nitrazepam	Cerilliant N-906, Alltech 017933
Nordiazepam	Cerilliant N-905, Alltech 013453
Oxazepam	Cerilliant O-902, Alltech 013703
Temazepam	Cerilliant T-907, Alltech 013833
Triazolam	Cerilliant T-910, Alltech 014283
α-Hydroxytriazolam	Cerilliant T-911

2.3.4.5.2 Non-extracted Standards

2.3.4.5.2.1 Run necessary NES analytical standards as indicated by examination of GC/MSD data.

2.3.4.5.2.2 Dilute 1.0mg/mL standard solution to 250ug/mL with methanol.

2.3.4.5.3 Non-extracted Derivatized Standards

2.3.4.5.3.1 Prepare a minimum of Oxazepam, Temazepam, Nordiazepam and Lorazepam TMS standards. Additional standards should be prepared as necessary indicated by current drug therapy or examination of GC/MSD data.

2.3.4.5.3.2 Add 10μL of stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.4.6.5.

2.3.4.5.4 Extracted Positive Controls (EPC)

2.3.4.5.4.1 Liquid Urine Control containing a minimum of Oxazepam or Nordiazepam (BioRad 443, Utak 88121 or equivalent)

2.3.4.5.4.2 Drug Mix Control

Drug Mix: Drug Mix (Alltech 601826 {Medazepam, Oxazepam, Lorazepam, Diazepam, Temazepam, and Bromazepam} or similar)

Negative Urine: Ansys 170A, Utak 88121-CDF (L) or equivalent.

Preparation: Pipette 10μL of drug mixture into 5mL of negative urine.

2.3.4.5.4.3 Enzyme Controls

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Conjugated Standard: Oxazepam Glucuronide (Alltech 01541 or equivalent).
Negative Urine: Ansys 170A, Utak 88121-CDF (L) or equivalent.
Preparation: Pipette 10μL of conjugated standard into 5mL of negative urine. Prepare two controls to allow for both the presence and absence of glucuronidase.

2.3.4.5.5 Extracted Negative Control

Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.3.4.6 PROCEDURE

2.3.4.6.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, tapered-bottom derivatization tubes and GC/MS vials with microinserts as follows for the negative control, positive controls, Oxazepam Glucuronide controls (with and without glucuronidase) and appropriate laboratory numbers without prefix. Label tapered-bottom derivatization tubes and GC/MS vials with microinserts for NES derivatized standards.

2.3.4.6.3 Sample Preparation

- Transfer 5.0ml of urine specimen, negative urine or positive control to extraction tube.

2.3.4.6.4 Sample Hydrolysis

- To each extraction tube add:
  - 2 ml β-Glucuronidase solution (pH 5.0)
- Cap and vortex *gently* to mix.
- Place in 65°C laboratory oven or waterbath for three hours.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Allow samples to cool.

2.3.4.6.5 Extraction

- Insert labeled 200mg CLEAN SCREEN® DAU column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate at ≤3 in. Hg (<10 kPa). .

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- Add 3mL of deionized water to the column and aspirate at ≤3 in. Hg (<10 kPa). .
- 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Decant sample into column and aspirate at <3 in. Hg.
- Wash column with 2mL of deionized water and aspirate at ≤ 3 in. Hg.
- Wash column with 2mL 20% acetonitrile in 0.1M phosphate buffer (pH 6.0) and aspirate and aspirate at ≤ 3 in. Hg.
- Increase vacuum to ≥10 in. Hg (≥34 kPa) and dry extraction disc for approximately 5 minutes.
- Wash column with 2mL hexane and aspirate and aspirate at ≤ 3 in. Hg.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 3mL ethyl acetate to elute the sample from the column at ≤ 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at ≤ 40°C.

2.3.4.7.6 Derivatization

In fume hood:

- Add 50 μL ethyl acetate.
- Add 50 μL silylating agent.
- Cap tubes with snap caps.
- Vortex.
- Heat tube for 20 minutes in 70°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MS ALS vial with microinsert.

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2.3.4.8 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

2.3.4.8.1 Analysis Parameters

2.3.4.8.1.1 Inject 1 μL into GC/MS using the ALS.

2.3.4.8.1.2 Analyze sample extract in full scan acquisition.

2.3.4.8.1.3 Refer to attached GC/MSD method printout for current analysis parameters.

2.3.4.8.2 Detection and Identification Criteria

2.3.4.8.2.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

- Acceptable retention time window is +/- 5%.

2.3.4.9 REFERENCES

2.3.3.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

2.3.3.9.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.4 Extraction of Benzodiazepines Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-18-02	Refinements

Approval

Technical Leader: ______________________ **Date:** __________
S. C. Williamson

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QC Manager: ______________________ **Date:** __________
Rick D. Groff

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.5 Extraction of Benzoylecgonine Employing the SPEC·PLUS™·DAU Extraction Column.

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2.3.5.1 BACKGROUND

Cocaine is a naturally occurring alkaloid derived from leaves of the South American shrub, Erythroxylon coca. Cocaine is also can be produced synthetically. Cocaine is one of the most potent stimulants to the central nervous system due to its mechanism of action, which involves blocking reuptake of stimulatory neurotransmitters. Cocaine is used licitly as a local anesthetic in ophthalmology. The positive effects of cocaine include an increased mental awareness and alertness, a sense of clarity and feelings of elation. The fictional detective Sherlock Holmes used cocaine for its transcendently stimulating and mind clarifying properties to the displeasure of Doctor Watson. As with all drugs, the effects of cocaine depend on the dosage, the form in which it is taken and the route of administration. Other significant factors include the setting or circumstances in which the drug is used and the expectations of the user. Side effects can include pupillary dilation, restlessness, dizziness, dyskinesia, tremor, dysphoria, and paranoia. Additional major side effects of cocaine use are a consequence of discontinued use. If the user does not readminister the drug, they may experience increased anxiety, agitation, restlessness and the disturbance of normal sleep patterns, which leads to fatigue. Due to these effects following cocaine use, an individual's ability to operate a motor vehicle is impaired both during and following cocaine use.

Routes of administration of cocaine include snorting, injection and smoking. The metabolism of cocaine and its metabolites involves hydrolysis, transesterification and n-demethylation. Cocaine metabolites detectable in urine include benzoylecgonine, ecgonine methyl ester, norcocaine and various arylhydroxy- and arylhydroxymethoxy-metabolites. The duration of the action of cocaine is limited by its rate of metabolism since its major metabolites are inactive. One of the active metabolites, cocaethylene is produced via transesterification when cocaine and ethanol are ingested concurrently.

2.3.5.2 PRINCIPLE

This procedure outlines the use of the SPEC·PLUS™ DAU SPE column for the extraction of Benzoylecgonine from urine. ANSYS Technologies' SPEC™ Solid Phase Extraction products are manufactured with polypropylene plastic and bonded-silica impregnated on a glass fiber disc. The DAU column utilizes a sorbent which combines a strong cation exchanger with a non-polar phase (reversed phase) to interact effectively, physically and chemically, with benzoylecgonine and minimally with interfering substances in the urine sample. The cation exchanger component of the phase is effective for compounds which are present in the urine in a cationic form. The sample pretreatment with 0.1M HCl ensures that the nitrogen group on the ecgonine portion of the cocaine molecule (pKa 8.6) bonds ionically to the sorbent. For the extraction of benzoylecgonine the urine is adjusted with a phosphate buffer to maximize the ionic character of the analyte. The sample is then applied to a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with the aqueous solvent, to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of solvent. When the column is dry, the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.5.3 EQUIPMENT AND SUPPLIES

2.3.5.3.1 SPEC·PLUS™ 3ml DAU column (Ansys 532-DAU or equivalent)

2.3.5.3.2 Drybath (Fisher or equivalent)

2.3.5.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.5.3.4 Vacuum Manifold/pump

2.3.5.3.5 <u>Glassware</u>

16X100 Test Tubes (Fisher 14-961-29 or equivalent)

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.5.3.6 pH paper (Fisher 09-876-17 or equivalent)

2.3.5.3.7 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g.

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100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

2.3.5.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.5.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.5.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.5.4.3 50% Methanol/water

2.3.5.4.4 0.1M Hydrochloric Acid

2.3.5.4.5 Elution Solvent

Mix 80mL ethyl acetate with 20mL methanol.

2.3.5.4.6 Silylating Agent (select from)

- MSTFA/1% TMCS (Pierce#48915 or equivalent)
- BSTFA/1% TMCS (Pierce#38831 or equivalent)

2.3.5.5 QUALITATIVE STANDARDS AND CONTROLS

2.3.5.5.1 Sources for Standards

Standard (in methanol)	*Potential Vendors*
Benzoylecgonine	Cerilliant B-004, Alltech 018203

2.3.5.5.2 Non-extracted Derivatized Standards (NES)

2.3.5.5.2.1 Add 10µL of benzoylecgonine stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.5.7.2.

2.3.5.5.3 Extracted Positive Controls

Liquid urine control which contains benzoylecgonine in the appropriate concentrations. (UTAK 66812-C or equivalent)

2.3.5.5.4 Extracted Negative Control

Negative urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.3.5.6 PROCEDURE

2.3.5.6.1 Initial set-up

Label the test tubes and GC/MS vials with microinserts for Negative Urine Control, Positive Urine Control and Appropriate Laboratory Numbers.

Label tapered tip centrifuge tubes for Negative Urine Control, Positive Urine Control, NES and Appropriate Laboratory Numbers.

2.3.5.6.2 Extraction Procedure Utilizing SPEC·PLUS™·DAU Extraction Column

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2.3.5.6.2.1 Transfer 1mL of urine specimen, Negative Control or Positive Control to an appropriate labeled test tube.
2.3.5.6.2.2 Add 1mL 0.1M HCl and Vortex.
2.3.5.6.2.3 Insert labeled SPEC·PLUS™·DAU extraction column into vacuum manifold
2.3.5.6.2.4 Add 200µL of methanol to column and wait for one minute.
2.3.5.6.2.5 Pour sample onto column and aspirate at 3-5 in. Hg (10-17 kPa)
2.3.5.6.2.6 Add 500µL of 0.1M HCl to column and aspirate at 3-5 in. Hg (10-17 kPa).
2.3.5.6.2.7 Add 500µL of 50% methanol/water to column and aspirate at 3-5 in. Hg (10-17 kPa).
2.3.5.6.2.8 Increase vacuum to 10-20 in. Hg. (34-68 kPa) and dry extraction disc for ≥5 minutes.
2.3.5.6.2.9 Open vacuum manifold, wipe collection tips, and insert collection holding rack containing the 16X144mm tapered tip centrifuge tubes.
2.3.5.6.2.10 Add 800µL of elution solvent to column and aspirate slowly at < 3 in. Hg (10kPa).
2.3.5.6.2.11 Increase vacuum to 5 in. Hg (17 kPa) to assist final amount of elution solvent through the disc.
2.3.5.6.2.12 Remove collection vials with elutes from rack.
2.3.5.6.2.13 Evaporate solvent to dryness under a gentle stream of nitrogen at approximately 60°C.
2.3.5.6.2.14 Add 25µL of ethyl acetate.
2.3.5.6.2.15 In the hood add 25µL of silylating agent.
2.3.5.6.2.16 Cap.
2.3.5.6.2.17 Vortex.
2.3.5.6.2.18 Heat for 15 minutes at 90°C.
2.3.5.6.2.19 Cool to room temperature
2.3.5.6.2.20 Transfer to the appropriately labeled ALS vial.

2.3.5.6.3 Automated Extraction Procedure Utilizing SPEC·PLUS™·DAU Extraction Column

2.3.5.6.3.1 Refer to the following attached methods/printouts.

2.3.5.6.4 Gas Chromatography/Mass Spectrometry (GC/MSD) Analysis

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2.3.5.6.4.1 Inject 1μL into GC/MSD using the ALS.

2.3.5.6.4.2 Analyze sample extract in full scan acquisition. Refer to attached GC/MSD method printout for current analysis parameters.

2.3.5.6.5 Detection and Identification Criteria

2.3.5.6.5.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus that of an authenticated standard.

2.3.5.6.5.2 Acceptable retention time window is ±5%.

2.3.5.7 REFERENCES

2.3.5.7.1 Automated SPEC® · Solid Phase Extraction Protocols for Drugs of Abuse Using the RapidTrace™ SPE Workstation, ANSYS, 1997.

2.3.5.7.2 SPEC·PLUS™·3ML DAU Drugs of Abuse in Urine Extraction Applications, Ansys, 1999

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.5 Extraction of Benzoylecgonine Employing the SPEC·PLUS™·DAU Extraction Column.

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Idaho State Police
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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.6 Extraction of Benzoylecgonine Employing United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column.

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2.3.6.1 BACKGROUND

Cocaine is a naturally occurring alkaloid derived from leaves of the South American shrub, Erythroxylon coca. Cocaine is also can be produced synthetically. Cocaine is one of the most potent stimulants to the central nervous system due to its mechanism of action, which involves blocking reuptake of stimulatory neurotransmitters. Cocaine is used licitly as a local anesthetic in ophthalmology. The positive effects of cocaine include an increased mental awareness and alertness, a sense of clarity and feelings of elation. The fictional detective Sherlock Holmes used cocaine for its transcendently stimulating and mind clarifying properties to the displeasure of Doctor Watson. As with all drugs, the effects of cocaine depend on the dosage, the form in which it is taken and the route of administration. Other significant factors include the setting or circumstances in which the drug is used and the expectations of the user. Side effects can include pupillary dilation, restlessness, dizziness, dyskinesia, tremor, dysphoria, and paranoia. Additional major side effects of cocaine use are a consequence of discontinued use. If the user does not readminister the drug, they may experience increased anxiety, agitation, restlessness and the disturbance of normal sleep patterns, which leads to fatigue. Due to these effects following cocaine use, an individual's ability to operate a motor vehicle is impaired both during and following cocaine use.

Routes of administration include snorting, injection and smoking. The metabolism of cocaine and its metabolites involves hydrolysis, transesterification and n-demethylation. Cocaine metabolites detectable in urine include benzoylecgonine, ecgonine methyl ester, norcocaine and various arylhydroxy- and arylhydroxymethoxy- metabolites. The duration of the action of cocaine is limited by its rate of metabolism since its major metabolites are inactive. One of the active metabolites, cocaethylene is produced via transesterification when cocaine and ethanol are ingested concurrently.

2.3.6.2 **PRINCIPLE**

This procedure outlines the use of the 200mg CLEAN SCREEN® DAU SPE column for the extraction of cocaine and benzoylecgonine from urine. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the urine sample.

The cation exchanger will allow the anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] The cation exchanger component of the phase is effective for compounds which are present in the urine sample in a cationic form bonding ionically to the sorbent.

For the extraction of cocaine and benzoylecgonine, the urine is adjusted to pH 6 with a phosphate buffer to maximize the ionic character of the analyte, and loaded onto a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the amine functional groups present on the drug and the anionic sulfonic acid exchanger on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

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2.3.6.3 **EQUIPMENT AND SUPPLIES**

2.3.6.3.1 200 mg CLEAN SCREEN® Extraction Column (ZSDAU020 OR ZCDAU020 or equivalent)

2.3.6.3.2 Drybath (Fisher or equivalent)

2.3.6.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.6.3.4 Vacuum Manifold/pump

2.3.6.3.5 Glassware

16X100 Test Tubes (Fisher 14-961-29 or equivalent)

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.6.3.6 pH paper (Fisher 09-876-17 or equivalent)

2.3.6.3.7 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

2.3.6.3 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.6.4.1 Methylene Chloride (Fisher D37-4 or equivalent)
2.3.6.4.2 Isopropanol (Fisher A416-1 or equivalent)
2.3.6.4.3 Ammonium Hydroxide (Fisher A669-500 or equivalent)
2.3.6.4.4 Methanol (Fisher A412-4 or equivalent)
2.3.2.4.5 Ethyl Acetate (Fisher E145-4 or equivalent)
2.3.6.4.6 Deionized/distilled (DI) water
2.3.6.4.7 100mM Phosphate buffer pH 6.0
2.3.6.4.8 100mM HCl
2.3.6.4.9 Elution Solvent
Mix 70mL methylene chloride, 20mL isopropyl alcohol, and 2mL ammonia hydroxide.
2.3.6.4.10 Silylating Agent (select from)

- MSTFA/1% TMCS (Pierce#48915 or equivalent)
- BSTFA/1% TMCS (Pierce#38831 or equivalent)

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2.3.6.5 QUALITATIVE STANDARDS AND CONTROLS

2.3.6.5.1 Sources for Standards

Standards (in solution)	*Potential Vendors*
Benzoylecgonine	Cerilliant B-004, Alltech 018203
Cocaine	Cerilliant C-008, Alltech 018003
Ecgonine methyl ester	Cerilliant E-001, Alltech 014553
Norcocaine	Cerilliant N-005, Alltech 6015353
Cocaethylene	Cerilliant C-010, Alltech 6015363
Norcocaethylene	Cerilliant N-024

2.3.6.5.2 Non-extracted Standards

2.3.6.5.2.1 Run necessary analytical standards as indicated by examination of GC/MSD data.

2.3.6.5.2.2 Dilute 1.0mg/mL standard solution to 250ug/mL with methanol.

2.3.6.5.3 Non-extracted Derivatized Standard (NES)

2.3.6.5.3.1 Add 10µL of benzoylecgonine stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.6.7.2.

2.3.6.5.4 Extracted Positive Controls

Liquid Urine Control which contains benzoylecgonine in the appropriate concentrations. (UTAK 66812-C or equivalent)

2.3.6.5.5 Extracted Negative Control

Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.3.6.6 PROCEDURE

2.3.6.6.1 Initial set-up

Label the test tubes and GC/MS vials with microinserts for Negative Urine Control, Positive Urine Control and Appropriate Laboratory Numbers.

Label tapered tip centrifuge tubes for Negative Urine Control, Positive Urine Control, NES and Appropriate Laboratory Numbers.

2.3.6.6.2 Extraction Procedure Utilizing 200mg CLEAN SCREEN® DAU Column

2.3.6.6.2.1 Transfer 5mL urine specimen, Negative Control or Positive Control to an appropriate labeled test tube.

2.3.6.6.2.2 Add 2mL 100mM phosphate buffer and Vortex. pH should be 6.0 ± 0.5. Adjust pH as necessary with 100mM monobasic or dibasic sodium phosphate.

2.3.6.6.2.3 Insert labeled CLEAN SCREEN® extraction column into vacuum manifold.

2.3.6.6.2.4 Add 3mL of methanol to column and aspirate at ≤3 in. Hg to prevent sorbent drying.

2.3.6.6.2.5 Add 3mL of DI H_2O to column and aspirate and aspirate at ≤3 in. Hg.

2.3.6.6.2.6 Add 1mL 100mM phosphate buffer (pH 6.0) to column and aspirate at ≤3 in. Hg.

2.3.6.6.2.7 Load sample onto column at 1 to 2 mL/minute.

2.3.6.6.2.8 Wash column with 2mL DI H_2O and aspirate at ≤3 in. Hg.

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2.3.6.6.2.9 Wash column with 2mL of 100mM hydrochloric acid and aspirate at ≤3 in. Hg.

2.3.6.6.2.10 Wash column with 3mL of methanol and aspirate at ≤3 in. Hg.

2.3.6.6.2.11 Dry column by aspirating at ≥ 10 in. Hg for ≥ 5 minutes.

2.3.6.6.2.12 Open vacuum manifold, wipe collection tips, and insert collection holding rack containing the 16X144mm tapered tip centrifuge tubes.

2.3.6.6.2.13 Add 3mL of elution solvent to column and aspirate slowly, < 3 in. Hg (10kPa).

2.3.6.6.2.14 Remove collection vials with elutes from rack.

2.3.6.6.2.15 Evaporate to dryness under a gentle stream of nitrogen at ≤ 40°C.

2.3.6.6.2.16 Add 50μL ethyl acetate.

2.3.6.6.2.17 In fume hood, add 50μL silylating agent.

2.3.6.6.2.18 Cap.

2.3.6.6.2.19 Vortex.

2.3.6.6.2.20 Heat for 20 minutes 70°C dry bath.

2.3.6.6.2.21 Remove from dry bath and cool to room temperature.

2.3.6.6.2.22 Transfer to the appropriately labeled ALS vial.

2.3.6.7.3 Automated Extraction Procedure Utilizing 200mg CLEAN SCREEN® extraction column.

2.3.6.7.3.1 Refer to the following attached methods/printouts.

2.3.6.7.4 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

2.3.6.7.4.1 Inject 1 μL into GC/MS using the ALS.

2.3.6.7.4.2 Analyze sample extract in full scan acquisition. Refer to GC/MSD method printout for current analysis parameters.

2.3.6.7.5 Detection and Identification Criteria

2.3.6.7.5.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus that of an authenticated standard.

2.3.6.7.5.2 Acceptable retention time window is ±5%.

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2.3.6.8 REFERENCES

2.3.6.8.1 UCT CLEAN SCREEN® Extraction Columns Application Manual.

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.6 Extraction of Benzoylecgonine Employing United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column.

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QC Manager: ______________________ **Date:** __________
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.7 Extraction of Codeine and Morphine Employing the SPEC·PLUS™·DAU Extraction Column.

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2.3.7.1 BACKGROUND

Morphine and codeine are natural derivatives of the opium poppy, *Papaver somiferum*. Opium contains several alkaloids including morphine, codeine and papaverine. Codeine is the phenolic methyl ether of morphine while heroin is a diacetyl derivative. Classified as opiates, codeine and morphine are used therapeutically primarily as analgesics and antitussives. While both morphine and codeine have potential for abuse, the illicit drug, heroin continues to be the second most widely abused drug in the United States (DAWN 2000). The effect these opiates exhibit is dependent upon their interactions with specific receptor sites within the central nervous system (CNS). In addition to analgesia and cough suppression, effects of opiate use include euphoria, respiratory depression, sedation, reduced GI motility/constipation, hypothermia, dysphoria, miosis, bradycardia, nausea, and physical tolerance and dependence.

Compound	Trade Name	Receptor/Action	Metabolites
Codeine	Tylenol 3®	μ agonist, δ agonist	morphine, norcodeine
Morphine	MSIR Roxanol MS Contin	μ agonist, κ agonist, δ agonist	minor quantities normorphine
Heroin	---------	μ agonist, κ agonist, δ agonist	6-monoacetylmorphine, morphine, small quantities of codeine (addicts)

2.3.7.2 PRINCIPLE

This procedure outlines the use of the ANSYS® Diagnostics, Inc SPEC·PLUS™ 3ml SPE column for the extraction of codeine and morphine from urine. ANSYS Technologies' SPEC™ Solid Phase Extraction products are manufactured with polypropylene plastic and bonded-silica impregnated on a glass fiber disc. The DAU column utilizes a sorbent which combines a strong cation exchanger with a non-polar phase (reversed phase) to interact effectively, physically and chemically, with benzoylecgonine and minimally with interfering substances in the urine sample. The cation exchanger component of the phase is effective for compounds which are present in the urine in a cationic form. Codeine and

morphine form glucuronide conjugates to facilitate their excretion. Prior to extraction, an enzymatic hydrolysis is required to free them from the glucuronide sugar moiety. For the extraction of codeine and morphine, the urine is adjusted with a low pH acetate buffer to maximize the ionic character of the analytes and the sorbent. The sample is then applied to a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with the aqueous solvent, to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of solvent. When the column is dry, the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

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2.3.7.3 EQUIPMENT AND SUPPLIES

2.3.7.3.1 SPEC·PLUS™·DAU extraction column. (Ansys 532-DAU or equivalent)

2.3.7.3.2 Drybath (Fisher or equivalent)

2.3.7.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.7.3.4 Vacuum Manifold/pump

2.3.7.3.5 <u>Glassware</u>
16X100 Test Tubes (Fisher 14-961-29 or equivalent)
16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)
Snap Caps (Fisher 05-538-41N or equivalent)
GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.7.3.6 pH paper (Fisher 09-876-17 or equivalent)

2.3.7.3.7 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

2.3.7.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.7.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.7.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.7.4.3 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.7.4.4 1.0M Acetate Buffer (pH 3.8)

2.3.7.4.5 0.1M Acetic Acid

2.3.7.4.6 1.5M Acetic Acid

2.3.7.4.7 1.5M Phosphate Buffer (pH 10.8)

2.3.7.4.8 Elution solvent
Mix 80mL of ethyl acetate, 20mL methanol and 2 mL ammonium hydroxide.

2.3.7.4.9 β-Glucuronidase Options
- Prepare from Patella vulgata Type L-II powder (Sigma G8132 or equivalent)
- Prepared Helix pomatia Type H-2 Solution (Sigma G0876 or equivalent)

2.3.7.4.10 Silylation Reagent Options
- MSFTA (Pierce 48910 or equivalent)

2.3.7.5 QUALITATIVE STANDARDS AND CONTROLS

2.3.7.5.1 Sources of Standards

Standards (in solution)	*Potential Vendors*
6-Acetylmorphine	Cerilliant A-009
Codeine	Cerilliant C006, Alltech 018013
Dihydrocodeine	Cerilliant D-019, Alltech 017773
Fentanyl	Cerilliant F-013, Alltech 013993
Heroin	Cerilliant H-038, Alltech 013653
Hydrocodone	Cerilliant H-003
Hydromorphone	Cerilliant H-004, Alltech 013553
Methadone	Cerilliant M-007, Alltech 018023
Morphine	Cerilliant M-005, Alltech 018033
Oxycodone	Cerilliant O-008, Alltech 013543
Oxymorphone	Cerilliant O-004, Alltech 013983

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2.3.7.5.3 Non-extracted Derivatized Standards

2.3.7.5.3.1 Prepare a minimum of Morphine, Codeine, and Hydrocodone TMS standards. Additional standards should be prepared as necessary indicated by current drug therapy or examination of GC/MSD data.

2.3.7.5.3.2 Add 10μL of stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.7.6.5.

2.3.7.5.4 Extracted Positive Controls (EPC)

2.3.7.5.4.1 Liquid Urine Control containing a minimum of Morphine and/or Codeine (BioRad 478, Utak 66812-C or equivalent)

2.3.7.5.4.2 Drug Mix Control

Drug Mix: Alltech 601827 {Codeine, Morphine, Hydromorphone, Oxycodone, Nalorphine and Diacetylmorphine} or equivalent

Negative Urine: Provided by FS employees, Ansys 170A, Utak 88121-CDF(L) or equivalent.

Preparation: Pipette 10μL of drug mixture into 5mL of negative urine.

2.3.7.5.4.3 Enzyme Controls

Conjugated Standard: Morphine-3β-D-glucuronide (Alltech [1mg/ml] M-031, [100μg/ml] M-018, or equivalent)

Negative Urine: Provided by FS employees, Ansys 170A, Utak 88121-CDF(L) or equivalent.

Preparation: Pipette 10μL of conjugated standard into 5mL of negative urine. Prepare two controls to allow for both the presence and absence of glucuronidase.

2.3.7.5.5 Extracted Negative Control

Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

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2.3.7.6 PROCEDURE

2.3.7.6.1 Initial set-up

Label SPEC·PLUS™·DAU extraction column, test tubes, tapered-bottom derivatization tubes and GC/MS vials with microinserts as follows for derivatized extractions (TMS) for the negative control, positive controls, Morphine-3β-D-glucuronide controls (with and without glucuronidase) and appropriate laboratory numbers without prefix. Label tapered-bottom derivatization tubes and GC/MS vials with microinserts for NES derivatized standards.

2.3.7.6.2 Sample Preparation

- Transfer 1.0mL urine specimen, negative urine or positive control to extraction tube.
- Add 200μL of 1.0M acetate buffer (pH 3.8)
- Vortex.
- pH should be approximately 4, adjust if necessary using 0.1M acetic acid or KOH.

2.3.7.6.3 Sample Hydrolysis

- To each extraction tube add:

- 200 µl β-Glucuronidase solution
- Cap and vortex *gently* to mix.
- Place in 60°C laboratory oven or waterbath for two hours.
- Allow samples to cool
- Add 1.0mL of phosphate buffer (pH 10.8)
- The resulting pH should be approximately 9.
- Adjust pH as necessary.
- Centrifuge for 5 minutes at 3000-3500 rpm

2.3.7.6.4 Extraction

- Insert labeled SPEC·PLUS™·DAU extraction column in the vacuum manifold.
- Add 200µL of methanol to the column and wait for one minute.
- Decant sample into column and aspirate at 3-5 in. Hg (10-17kPa).
- Wash column with the following and aspirate aspirate at 3-5 in. Hg (10-17kPa)
  - 500µL of deionized water.
  - 500µL 0.1M acid.
  - 500µL methanol
- Increase vacuum to 10-20 in. Hg (34-68 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 800µL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa) to aspirate solvent through disc into collection vial.
- Increase vacuum to 5in. Hg (17kPa) to assist final amount of elution solvent through disc.
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at approximately 60°C.

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2.3.7.6.5 Derivatization

- In fume hood, add 50µL of silylating agent and 50µL ethyl acetate.
- Cap tubes with snap caps.
- Vortex.
- Heat tube for 20 minutes in 60°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

2.3.7.6.6 Automated Extraction Procedure Utilizing SPEC·PLUS™ - 3ml DAU column.

2.3.7.6.6.1 Refer to the following attached method/printouts.

2.3.7.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

2.3.7.7.1 Analysis Parameters

2.3.8.7.1.1 Inject 1 µL into GC/MSD using the ALS.

2.3.8.7.1.2 Analyze sample extract in full scan acquisition.

2.3.8.7.1.3 Refer to attached GC/MSD method printout for current analysis parameters.

2.3.7.7.2 Detection and Identification Criteria

2.3.8.7.2.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

- Acceptable retention time window is +/- 5%.

2.3.7.8 REFERENCES

2.3.7.8.1 Automated SPEC® · Solid Phase Extraction Protocols for Drugs of Abuse Using the RapidTrace™ SPE Workstation, ANSYS, 1997.

2.3.7.8.2 SPEC·PLUS™·3ML·DAU Drugs of Abuse in Urine Extraction Applications, Ansys, 1999.

2.3.7.8.3 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man 5th ed., Chemical Toxicology Institute, 2000.

2.3.7.8.4 Hutchison TA & Shahan DR (Eds): DRUGDEX® System. MICROMEDEX, Inc., Greenwood Village, Colorado (Edition expires [12/01]).

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.7 Extraction of Codeine and Morphine Employing the SPEC·PLUS™·DAU Extraction Column.

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-17-02	Refinements

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Approval

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S. C. Williamson

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QC Manager: ______________________ **Date:** ____________
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.8 Extraction of Opiates Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Extraction Column.

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2.3.8.1 BACKGROUND

Morphine and codeine are natural derivatives of the opium poppy, *Papaver somiferum*. Opium contains several alkaloids including morphine, codeine and papaverine. These natural products lead to the development of numerous synthetic analgesics. Narcotic analgesics are divided into 3 classes, the phenanthrenes (morphine, codeine, oxycodone, pentazocine), phenylpiperidines (meperidine, fentanyl), and the phenylheptanes (methadone, propoxyphene). As illustrated in the chart below, the effects of opiate class drugs are dependent upon interactions with specific receptor sites within the central nervous system (CNS). In addition to analgesia and cough suppression, effects of opiate use include euphoria, respiratory depression, sedation, reduced GI motility/constipation, hypothermia, dysphoria, miosis, bradycardia, nausea, and physical tolerance and dependence.

Compound	Trade Name	Receptor/ Action	Metabolites	Therapeutic uses
Buprenorphine	Buprenex®	μ agonist, κ antagonist	Norbuprenorphine	moderate-severe pain
Butorphanol	Stadol®, Stadol NS®	κ agonist, μ antagonist	3-hydroxybutorphanol, norbutorphanol	moderate-severe pain
Codeine	Tylenol 3®	μ agonist, δ agonist	morphine, norcodeine	mild-moderate
Dihydrocodeine	Paracodin®	μ agonist	dihydromorphine, nordihydrocodeine	mild-moderate
Fentanyl	Sublimaze®	μ agonist	despropionylfentanyl, norfentanyl, hydroxyfentanyl, hydroxynorfentanyl	moderate-severe
Heroin	NA in US	μ agonist	6-acetylmorphine, morphine, normorphine	----
Hydrocodone	Hycodan®, Vicodin®, Codone®, Lortab®	μ agonist	hydromorphone, norhydrocodone, dihydrocodeine hydromorphol	moderate-severe
Hydromorphone	Dilaudid®	μ agonist	hydromorphol	moderate-severe
Levorphanol	levo-dromoran®	μ agonist, κ agonist	norlevorphanol	moderate-severe
Meperidine	Demerol®	μ agonist	normeperidine	moderate-severe

Compound	Trade Name	Receptor/ Action	Metabolites	Therapeutic uses
Methadone	Dolophine®, Methadose®	μ agonist	methadol, normethadol, EDDP, EMDP	Detoxification
Morphine	MS-IR Roxanol	μ agonist, κ agonist, δ agonist	normorphine	moderate-severe
Nalbuphine	Nubain ®	κ agonist, σ agonist, μ antagonist	nornalbuphine	moderate-severe
Oxycodone	Percolone®, Roxicodone®, Oxycontin®, Oxy®	μ agonist	oxymorphone, noroxycodone	moderate-severe
Oxymorphone	Numorphan®	μ agonist	6-oxymorphol	moderate-severe
Pentazocine	Talwin®	μ agonist, κ agonist, σ agonist	cis- and trans-hydroxypentazocine, trans-carboxypentazocine	moderate-severe
Propoxyphene	Darvon®, Darvocet®	μ agonist	norpropoxyphene,	mild-moderate
Tramadol	Ultram®	μ agonist	nortramadol, O-desmethyltramadol, N-desmethyltramadol	moderate

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2.3.8.2 PRINCIPLE

This procedure outlines the use of the UCT 200 mg CLEAN SCREEN® extraction column for the extraction of Opiates from urine. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the urine sample. The cation exchanger utilizes an anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

Opiates form glucuronide conjugates to facilitate their excretion. Prior to extraction, an enzymatic hydrolysis is required to free them from the glucuronide sugar moiety. For the extraction of opiates, the hydrolyzed urine is adjusted with a low pH acetate buffer, to maximize the ionic character of the analytes and the sorbent. The sample is then loaded onto a pre-conditioned SPE column. The conditioning creates an environment that allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the cationic functional groups present on the drug and the anionic sulfonic acid exchanger on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and

organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.8.3 EQUIPMENT AND SUPPLIES

2.3.8.3.1 200mg CLEAN SCREEN® extraction column (ZSDAU020 OR ZCDAU020 or equivalent)

2.3.8.3.2 Drybath (Fisher or equivalent)

2.3.8.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.8.3.4 Vacuum Manifold/pump

2.3.8.3.5 Glassware

16X100 Test Tubes (Fisher 14-961-29 or equivalent)

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.8.3.6 pH paper (Fisher 09-876-17 or equivalent)

2.3.8.3.7 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

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2.3.8.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.8.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.8.4.2 Methylene Chloride (Fisher D37-4 or equivalent)

2.3.8.4.3 Isopropanol (Fisher A416-1 or equivalent)

2.3.8.4.4 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.8.4.5 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.8.4.6 Deionized/distilled water

2.3.8.4.7 1.0M Acetate Buffer (pH 5.0)

2.3.8.4.8 100mM Phosphate Buffer (pH 6.0)

2.3.8.4.9 100mM Acetate Buffer (pH 4.5)

2.3.8.4.10 1N NaOH

2.3.8.4.11 Elution Solvent

Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***

2.3.8.4.12 β-Glucuronidase (Patella vulgata)

2.3.8.4.13 Silylation Reagent

BSTFA + 1% TMCS (UCT SBSTFA-1-1, Pierce 38831 or equivalent)

2.3.8.5 QUALITATIVE STANDARDS AND CONTROLS

2.3.8.5.1 Sources of Standards

Standards (in solution)	*Potential Vendors*
6-Acetylmorphine	Cerilliant A-009
Codeine	Cerilliant C006, Alltech 018013
Dihydrocodeine	Cerilliant D-019, Alltech 017773
Fentanyl	Cerilliant F-013, Alltech 013993
Heroin	Cerilliant H-038, Alltech 013653
Hydrocodone	Cerilliant H-003
Hydromorphone	Cerilliant H-004, Alltech 013553
Methadone	Cerilliant M-007, Alltech 018023
Morphine	Cerilliant M-005, Alltech 018033
Oxycodone	Cerilliant O-008, Alltech 013543
Oxymorphone	Cerilliant O-004, Alltech 013983

2.3.8.5.3 Non-extracted Derivatized Standards

2.3.8.5.3.1 Prepare a minimum of Morphine, Codeine, and Hydrocodone TMS standards. Additional standards should be prepared as necessary indicated by current drug therapy or examination of GC/MSD data.

2.3.8.5.3.2 Add 10μL of stock solution to labeled tapered bottom centrifuge tube. Derivatize as described in 2.3.8.6.5.

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2.3.8.5.4 Extracted Positive Controls

2.3.8.5.4.1 Liquid Urine Control containing a minimum of Morphine and/or Codeine (BioRad 478, Utak 66812-C or equivalent)

2.3.8.5.4.2 Drug Mix Control

Drug Mix: Alltech 601827 {Codeine, Morphine, Hydromorphone, Oxycodone, Nalorphine and Diacetylmorphine} or equivalent

Negative Urine: Ansys 170A, Utak 88121-CDF(L) or equivalent.

Preparation: Pipette 10μL of drug mixture into 5mL of negative urine.

2.3.8.5.4.3 Enzyme Controls

Conjugated Standard: Morphine-3β-D-glucuronide (Alltech [1mg/ml] M-031, [100μg/ml] M-018, or equivalent)

Negative Urine: Ansys 170A, Utak 88121-CDF(L) or equivalent.
Preparation: Pipette 10µL of conjugated standard into 5mL of negative urine. Prepare two controls to allow for both the presence and absence of glucuronidase.

2.3.8.5.5 Extracted Negative Control
Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.3.8.6 PROCEDURE

2.3.8.6.1 Initial set-up
Label 200mg CLEAN SCREEN® Extraction Column, test tubes, tapered-bottom derivatization tubes and GC/MS vials with microinserts as follows for derivatized extractions (TMS) for the negative control, positive controls, Morphine-3β-D-glucuronide controls (with and without glucuronidase) and appropriate laboratory numbers without prefix. Label tapered-bottom derivatization tubes and GC/MS vials with microinserts for NES derivatized standards.

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2.3.8.6.2 Sample Preparation
- Transfer 5.0mL of urine specimen, negative urine or positive controls to extraction tube.

2.3.8.6.3 Sample Hydrolysis
- To all extraction tubes, except the without enzyme control, add:
  - 2 mL β-Glucuronidase solution (pH 5.0)
- Cap and vortex *gently* to mix.
- Place in 65°C laboratory oven or waterbath for three hours.
- Allow samples to cool
- Centrifuge for 10 minutes at 2000 rpm and discard pellet
- Adjust pH to 6.0 ± 0.5 with approximately 700µl of 1.0N NaOH

2.3.8.6.4 Extraction
- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.

- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample into column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg
  - 2mL of deionized water
  - 2mL 100mM acetate buffer (pH 4.5)
  - 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 3mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at ≤ 40°C.

2.3.8.6.5 Derivatization

- In fume hood add the following:
  - 50µL ethyl acetate.
  - 50µL silylating agent.
- Cap tubes with snap caps.
- Vortex.
- Heat tube for 20 minutes in 70°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

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2.3.8.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

2.3.8.7.1 Analysis Parameters

2.3.8.7.1.1 Inject 1 µL into GC/MS using the ALS.

2.3.8.7.1.2 Analyze sample extract in full scan acquisition.

2.3.8.7.1.3 Refer to attached GC/MSD method printout for current analysis parameters.

2.3.8.7.2 Detection and Identification Criteria

2.3.8.7.2.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

- Acceptable retention time window is +/- 5%.

2.3.8.8 REFERENCES

2.3.8.8.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

2.3.8.8.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

2.3.8.8.3 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man 5th ed., Chemical Toxicology Institute, 2000.

2.3.8.8.4 Hutchison TA & Shahan DR (Eds): DRUGDEX® System. MICROMEDEX, Inc., Greenwood Village, Colorado (Edition expires [12/01]).

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.8 Extraction of Opiates Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Extraction Column.

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-17-02	Refinements

Approval

Technical Leader: ______________________ **Date:** __________
S. C. Williamson

Issuance

QC Manager: ______________________ **Date:** __________
Rick D. Groff

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.9 Qualitative Confirmation of Gamma Hydroxybutyrate in Urine Samples and GHB Containing Products

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2.3.9.1 BACKGROUND

This method provides a solid phase extraction option for the extraction of Gamma-hydroxybutyrate/Gamma-Hydroxybutyric Acid (GHB). GHB occurs naturally in minute quantities as a result of the metabolism of the inhibitory neurotransmitter, GABA.[6,8] The drug GHB is a potent tranquilizer that was previously used as an anesthetic and as a treatment for major depressive illnesses, alcohol withdrawal, and narcolepsy.[1-8] Legitimate use is limited due the side effects associated with the drug. GHB can produce visual disturbances, nausea, vomiting, drowsiness, dizziness, severe respiratory depression, unconsciousness and involuntary muscle spasms.[1,5,6] Overdoses can require emergency medical treatment including intensive care due to the respiratory depression, bradycardia and coma.[5,6,8]

The use of GHB has been increasing since the 1980s and currently is popular among body builders, teenagers and on the club/dance scene. Body builders use the drug due its alleged role as a growth hormone releasing agent to enhance muscle growth.[5,6,8] GHB has not been proven to possess any anabolic effects.[6] GHB has gained notoriety as a date rape drug due to its ability to produce short-term amnesia and decreased inhibitions.[1-8] The FDA banned the use of GHB in 1990 except for FDA approved physical supervised protocols.[6,8]

A dose of GHB consists of a capful that is usually approximately one teaspoon. This results in a dose anywhere from 2.5 to 4.0 grams of GHB. The taste of GHB has been described as salty or soapy, the odor is said to be mothball-like.[5,6] Due to the short half-life of GHB (0.3 to 1.0 hours[5], 27 ± 5 minutes[7]) the person will re-administer every 45 minutes to 1 hour. The onset of effects is 15 to 60 minutes. The effects of the drug will be detectable during a DRE exam for 4 to 6 hours. GHB is classified as a central nervous system depressant. The observed effects include horizontal and vertical nystagmus, lack of convergence, body tremors, and slowed breathing. The person will also exhibit a lowered pulse, blood pressure, and body temperature. In addition, the muscle tone will exhibit flaccidity and the person may be in a trance-like state, the pupils will exhibit a lack of reaction to light.[5] Lower doses will promote an agitated, combative state however their pulse and other vitals will be depressed.[5,6]

Combining GHB with alcohol plus a stimulant or marihuana allows the user to remain conscious during use. This allows them to experience the euphoric "buzz" that is the desired effect of its abuse. The desired effect is a state of relaxation and tranquility, a pleasant drowsiness, mild euphoria, hallucinations and a release of inhibitions. Combining GHB with alcohol or other central nervous system depressant will provide an additive depressant effect.[5]

GHB is detectable in blood for up to eight hours and in urine for up to 12 hours [5,6,7]. Peak plasma levels are obtained in 20 to 45 minutes.[7] Peak urine GHB concentrations on the order of 1100 μg/mL are observed within the first four hours after a 100 mg/kg oral dose.[3,7]

GHB is manufactured by reacting butyrolactone with sodium hydroxide in an aqueous solution in the presence of a low molecular weight alcohol (methanol, ethanol).[5,6] The average yield of GHB is 70%. If the yield of the manufacturing process is low, there will be significant amounts of the lactone present in the product. Abuse of this compound will continue due to its relatively simple synthesis and the availability and low cost of starting materials.[5] Users can ingest Gamma butyral lactone (GBL), a degreaser and floor stripper, and it is converted in vivo into GHB. GBL is therefore diverted from legitimate sources to either be taken directly or converted to GHB.

This compound has been referred to by a variety of names as listed in the table below.

Street Names	Marketing Names
"G"	Revitalize
"G" caps	Rejuvenate
Liquid X	Renewtrient
Soap	Revivarant
Easy Lay	Blue Nitro
	Thunder Nectar
	Rest-Eze
	Energy Drink

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There are thousands of documented GHB overdoses with numerous deaths. The danger in GHB use stems from its steep dose-response curve. A small increase in dose can create a dramatic difference in adverse effects. This makes the potential of overdosing with GHB very high. This is compounded by the fact that GHB effects users so differently. A dose that one individual uses could adversely effect another, thus word of mouth is a poor determiner of how much of the substance to use.

2.3.9.2 PRINCIPLE

GHB is extracted first into acetone, which is dried and reconstituted with phosphate buffer (pH 6.0). This sample extract is applied to a pretreated/conditioned SPE column. This method requires no heating to

create the GHB-TMS derivative. The SPE elutant is evaporated and a di-TMS derivative of GHB is prepared. The resulting derivative can be analyzed by either full scan or SIM GC/MSD in EI mode.

2.3.9.3 EQUIPMENT

2.3.9.3.1 Evaporative Concentrator (Zymark Turbo-Vap or equivalent).

2.3.9.3.2 Laboratory Centrifuge

2.3.9.3.3 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%diphenyl)

2.3.9.4 SUPPLIES

2.3.9.4.1 Glassware

Tapered tip 16X144 centrifuge tubes (Fisher catalog 05-538-41C or equivalent)

Snap caps (Fisher 05-538-41N or equivalent)

GC/MS vials (HP 5182-0865 or equivalent)

GC/MS vial microinserts (HP 5183-2088 or equivalent)

2.3.1.11.3.2 1.5mL snap cap centrifuge tubes (Fisher # or equivalent).

2.3.9.4.2 CLEAN SCREEN® GHB SPE columns (United Chemical Technologies, Inc., #ZSGHB020 or equivalent)

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2.3.9.5 REAGENTS

2.3.9.5.1 Hexane (Fisher #H303 or equivalent)

2.3.9.5.2 Sodium phosphate monobasic (Fisher #S369 or equivalent)

2.3.9.5.3 Sodium phosphate dibasic (Fisher #S374 or equivalent)

2.3.9.5.4 Dimethylformamide (Fisher #D119 or equivalent)

2.3.9.5.5 Ammonium hydroxide (Fisher #A669 or equivalent)

2.3.9.5.6 Methanol (Fisher #A454 or equivalent)

2.3.9.5.7 Ethyl Acetate (Ansys #203 or Fisher #E145-1 or equivalent)

2.3.9.5.8 Silylating Agent (select from)

BSTFA + 1% TMCS (UCT SBSTFA-1-1, Pierce 38831 or equivalent)

2.3.9.6 STANDARDS AND SOLUTIONS

Refer to section 2.6 for buffer solution preparation.

2.3.9.6.1 <u>GHB Stock Solution</u>
1.0mg/mL (Radian International #G-001 or equivalent)

2.3.9.6.2 <u>Extracted Negative Control</u>
Negative Urine (Ansys 170A, Utak 88121-CDF (L) or equivalent.)

2.3.9.6.3 0.1M Phosphate Buffer

2.3.9.6.4 99:1 $CH_3OH:NH_4OH$
Place ~80mL of methanol in a 100mL volumetric flask. Add 1mL of ammonium hydroxide, QS to 100mL.
Prepare fresh daily.

2.3.9.7 PROCEDURE

2.3.9.7.1 <u>Initial set-up</u>

2.3.9.7.1.1 Label GHB SPE extraction columns as follows:

- *GHB-NC* (Negative Control)
- *GHB-PC* (Positive Control)
- Laboratory numbers of samples without prefix.

2.3.9.7.1.2 Label two tapered-end centrifuge tubes and a GC/MS vial as follows:

- *GHB-NC* (Negative Control)
- *GHB-PC* (Positive Control)
- Laboratory numbers of samples without prefix.
- *GHB-NES* (Non-extracted GHB standard)

2.3.9.7.2 <u>Preparation of GHB Positive Controls and Standards</u>

2.3.9.7.2.1 <u>Spiked Urine</u> - GHB Positive Control [200μg/mL]
Add 50uL of 1mg/mL GHB stock to 200uL negative urine. Vortex.

2.3.9.7.2.2 <u>Non-Extracted Standard [200μg] (NES)</u>
Place 200uL of GHB stock into tapered-end centrifuge tube. Derivatize as described in 2.3.9.7.8.

2.3.9.7.3 <u>Extraction procedure</u>

2.3.9.7.3.1 To a tapered-end centrifuge tube add 200uL of specimen, negative or positive control urine.

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2.3.9.7.3.2 Add 1mL of acetone, vortex for 15 seconds.

2.3.9.7.3.3 Centrifuge tube at ≈3300rpm for 10 minutes.

2.3.9.7.3.4 Transfer solvent from tube into tapered-end centrifuge tube.

2.3.9.7.3.5 Evaporate solvent with nitrogen at 80°C in TurboVap apparatus.

2.3.9.7.3.6 Reconstitute the evaporated extracts with 200uL of 0.1 M phosphate buffer (pH 6.0). Vortex 15 seconds.

2.3.9.7.4 Column Conditioning

Prepare CLEAN SCREEN® GHB SPE column as follows:

2.3.9.7.4.1 Apply 3mL of MeOH; aspirate at ≤ 3 inches of Hg.

2.3.9.7.4.2 Apply 3mL of DI H2O; aspirate ≤ 3 inches of Hg.

2.3.9.7.4.3 Apply 3 mL of 0.1M Phosphate Buffer (pH 6.0), aspirate ≤ 3 inches of Hg.

2.3.9.7.5 Sample Application

Add sample to prepared column with air displacement pipet (MLA, Eppendorf). Aspirate at 1 inch Hg.

2.3.9.7.6 Collection of Extract

Place tapered bottom centrifuge tube into collection rack. Add 1mL of MeOH/NH_4OH (99:1) to original sample centrifuge tube (from step 6.3.5), vortex.
Decant onto column and collect extract.

2.3.9.7.7 Concentration of Extract

Place tubes from vacuum manifold into TurboVap apparatus. Evaporate solvent with nitrogen at 70°C.

2.3.9.7.8 Derivatization Procedure

2.3.9.7.8.1 Add 100µL of Ethyl acetate and 100µL of BSTFA with 1% TCMS to evaporated extracted samples, spiked standards and non-extracted standard.

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2.3.9.7.8.2 Transfer derivative to GC/MS ALS vial for analysis.

2.3.9.7.8.3 Inject 1 μL into GC/MS.

2.3.9.7.9 Gas Chromatography/Mass Spectrometry (GC/MS) Parameters

2.3.9.7.9.1 Oven program, Injector and Interface Temperatures:
Refer to Method print-out that follows SOP.

2.3.9.7.9.2 Sample should be analyzed in full scan acquisition. Refer to attached GC/MSD method print-out for current parameters.

2.3.9.7.10 Detection and Identification Criteria
The qualitative presence of GHB can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

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2.3.9.8 REFERENCES

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12.3.9.8.4 ElSohly, M.A. and Salamone, S.J. *Prevalence of Drugs used in Cases of Alleged sexual Assault* J Anal Tox, 1999, 23:141-146.

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Idaho State Police
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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.9 Qualitative Confirmation of Gamma Hydroxybutyrate in Urine Samples and GHB Containing Products

Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format
2	10-17-02	Refinements

Approval

Technical Leader: ______________________ **Date:** ____________
S. C. Williamson

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QC Manager: ______________________ **Date:** ____________
Rick D. Groff

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Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.10 General Operation and Maintenance of Zymark RapidTrace™ Station

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2.3.10.1 SCOPE

This method describes the general operation and maintenance to be performed on the Zymark RapidTrace™ Solid Phase Extraction (SPE) Workstation. This workstation allows for the automated processing of SPE columns.

2.3.10.2 EQUIPMENT

2.3.10.2.1 Zymark RapidTrace SPE Workstation

2.3.10.2.2 Glassware

- 12 X 75mm Culture tubes (Fractionation tube) (Fisher 14-961-26 or equivalent)
- 13 X 100mm (Sample tube) (Fisher 14-961-27 or equivalent)
- GC/MSD vials (HP 5182-0865 or equivalent)
- GC/MSD vial microinserts (HP 5183-2088 or equivalent)

2.3.10.2.3 Empty cartridges with spacers supplied with the RapidTrace™ startup kit

2.3.10.3 REAGENTS AND SOLUTIONS

Refer to manual section 2.6 for solution preparation instructions.

2.3.10.3.1 SPE Sample Preparation
Refer to specific method for reagents and solutions.

2.3.10.3.2 Post-run Clean-up
Distilled Water
2N Sodium Hydroxide
2N Nitric Acid

2.3.10.4 PROCEDURE

2.3.10.4.1 Initial set-up

2.3.10.4.1.1 Turn on RapidTrace™ instrument, computer, and gas. Ensure that gas pressure is between 10 psi and 40 psi.

2.3.10.4.1.2 Attain reagents and set-up dispenser lines as necessary for the particular SPE procedure. Make certain that there is sufficient reagent volume for the desired number of samples and lines are in the proper reservoir.

2.3.10.4.1.3 Check waste reservoirs.

2.3.10.4.1.4 <u>RapidTrace™ Software</u>
Open software by double clicking on the RapidTrace™ icon to open the menu, then double clicking on the RapidTrace™ production icon.

2.3.10.4.1.5 <u>Pre-run Instrument Line Purge</u>

- Place empty (dummy) cartridges with spacers in the SPE column turret.
- Place empty test-tubes in left and right portions of rack.
- Single click on the *SETUP RACKS* button.
- To select procedure for prime/purge, highlight by clicking on its procedure.
- Highlight sample one by clicking on it with the cursor.
- Click on the *ARROW* button to assign the highlighted procedure to the sample number.
- In the lower left, select the appropriate modules to run the procedure on.
- Select *OK/SAVE* button to exit to the main menu.
- Select the *RUN/MONITOR* button.
- Single click on the *RUN* button below each module window to run the procedure on that module.

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2.3.10.4.2 <u>General Operation</u>

2.3.10.4.2.1 Place samples into appropriately labeled 13 X 100mm test tubes. Place tube in the right side of the sample rack.

2.3.10.4.2.2 For each sample, place a corresponding fraction tube (12 X 75mm) in the left side of sample rack.

2.3.10.4.2.3 Place the appropriate type and number of SPE columns in the turret.

2.3.10.4.3 Automated Sample Extraction

2.3.10.4.3.1 Open the RapidTrace™ software.

2.3.10.4.3.2 Single click on the *SETUP RACKS* button.

2.3.10.4.3.3 Select the appropriate procedure by highlighting and clicking on it.

2.3.10.4.3.4 Highlight sample(s) by clicking on it with the cursor.

2.3.10.4.3.5 Click on the *ARROW* button to assign the highlighted procedure to that sample number.

2.3.10.4.3.6 In the lower left, select the appropriate modules to run the procedure on.

2.3.10.4.3.7 Select *OK/SAVE* button to exit to the main menu.

2.3.10.4.3.8 Select the *RUN/MONITOR* button.

2.3.10.4.3.9 Single click on the *RUN* button below each module window to run the procedure on that module.

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2.3.10.4.4 End of Day Clean-up

2.3.10.4.4.1 Place two sample tubes in the first two positions of the rack and the appropriate fraction tubes in corresponding locations.

2.3.10.4.4.2 Place two empty cartridges with spacers for sample one and two in the turret.

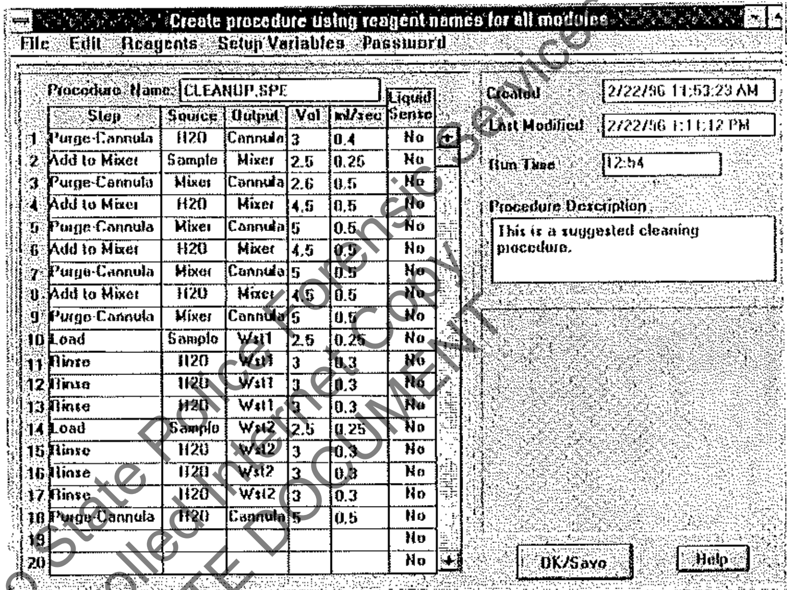
2.3.10.4.4.3 Place approximately 8 ml of 2 N Sodium Hydroxide (NaOH) into the first sample test tube.

2.3.10.4.4.4 Place approximately 8 ml of 2N Nitric Acid (HNO_3) into the second sample test tube.

2.3.10.4.4.5 Assign the appropriate cleaning procedure to these test tubes as described in 2.3.10.4.3.1 through 2.3.10.4.3.9.

2.3.10.4.4.6 This procedure is described on pages 46–47 of the RapidTrace™ workstation manual.

The workstation must be cleaned after each use to eliminate protein buildup. Illustrated below is a suggested cleanup procedure.



	Step	Source	Output	Vol	ml/sec	Liquid Sense
1	Purge-Cannula	H20	Cannula	3	0.4	No
2	Add to Mixer	Sample	Mixer	2.5	0.25	No
3	Purge-Cannula	Mixer	Cannula	2.6	0.5	No
4	Add to Mixer	H20	Mixer	4.5	0.5	No
5	Purge-Cannula	Mixer	Cannula	5	0.5	No
6	Add to Mixer	H20	Mixer	4.5	0.5	No
7	Purge-Cannula	Mixer	Cannula	5	0.5	No
8	Add to Mixer	H20	Mixer	4.5	0.5	No
9	Purge-Cannula	Mixer	Cannula	5	0.5	No
10	Load	Sample	Wst1	2.5	0.25	No
11	Rinse	H20	Wst1	3	0.3	No
12	Rinse	H20	Wst1	3	0.3	No
13	Rinse	H20	Wst1	3	0.3	No
14	Load	Sample	Wst2	2.5	0.25	No
15	Rinse	H20	Wst2	3	0.3	No
16	Rinse	H20	Wst2	3	0.3	No
17	Rinse	H20	Wst2	3	0.3	No
18	Purge-Cannula	H20	Cannula	5	0.5	No
19						No
20						No

2.3.10.5 REFERENCES

2.3.10.5.1 RapidTrace™ workstation manual- *SPE Workstation Installation and Quick Reference Guide*, Zymark® Corporation, Rev 2

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Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation
2.3.10 General Operation and Maintenance of Zymark RapidTrace™ Station

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Revision #	Issue Date	History
1	02-05-02	Original Issue in SOP format

Approval

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S. C. Williamson

Issuance

QC Manager: ______________________ **Date:** ____________
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Two
2.4 Liquid-Liquid Extraction Methods for GC/MSD Confirmation
2.4.1 General Extraction of Urine Samples for Qualitative Confirmation of Basic and Neutral or Acidic Drugs

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2.4.1.1 BACKGROUND

These extraction procedures are extensions of the TOXI-LAB® TOXI-A and TOXI-B thin layer chromatography (TLC) drug detection systems. The samples are extracted as with the TLC system, however, instead of concentrating the extract onto a disc, the solvent extract is concentrated and placed into an automated liquid sampler (ALS) vial for analysis by a gas chromatograph equipped with a mass selective detector (GC/MSD).

2.4.1.2 PRINCIPLE

This procedure describes the extraction of drug compounds from urine. Depending upon the pK_a of a drug compound, either Toxi-A or Toxi-B tubes are used. Basic compounds are extracted with a Toxi-A tube. Addition of urine to the Toxi-A tubes results in the urine becoming alkaline (pH=9) into 1,2-Dichloroethane, dichloromethane, heptane and isopropanol. Acidic compounds are isolated from an acidified solution (pH=4.5) into methylene chloride and heptane with zinc chloride to facilitate the extraction process. The extraction is achieved with an Ansys Toxi-B extraction tube. Either resulting extract is analyzed by full scan GC/MS in EI mode.

2.4.1.3 EQUIPMENT AND SUPPLIES

2.4.1.3.1 Tube Rocker (Fisher Scientific or equivalent)

2.4.1.3.2 Electric Warmer with Omega-12 extraction solvent concentrator (Ansys 118/153)

2.4.1.3.3 Laboratory Centrifuge (Fisher Marathon or equivalent)

2.4.1.3.4 Disposable Aluminum Concentration Cups (Ansys 152)

2.4.1.3.5 <u>Glassware</u>
GC/MS vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.4.1.3.6 Gas Chromatograph equipped with a mass selective detector (HP 6890/5973) and a HP-5MS Ultra low bleed (5%-Diphenyl-95%-Dimethylsiloxane copolymer) capillary column (25M).