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

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- *Refer to Separate Binder*

1.1 BACKGROUND

1.1.1 Principle of ELISA Analysis

ELISA is an acronym for enzyme-linked immunosorbent assay. An ELISA is an immunoassay in which one reactant is immobilized on a solid phase and the signal generator is an enzyme. The enzyme delivers a signal to indicate a particular antigen-antibody reaction has occurred and to what extent. 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.

1.1.2 Description of PersonalLAB™ BioChem ImmunoSystems Instrumentation

The PersonalLAB™ 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. The photometer reads the plate vertically. When the procedure is complete the PersonalLAB™ records the resulting absorbances. The instrumental software allows for the proper identification of samples.

1.1.3 Description of OraSure Technologies Micro-plate Assay

1.1.3.1 Intended Use

OraSure Micro-plate assay kits are intended for use in the qualitative determination of drugs-of-abuse in blood and urine. As described in the package inserts for each specific assay kit, the result of the assay is intended as only a preliminary analytical test result.

1.1.3.2 Overview of Assay

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 assays requires a predilution step which dilutes the 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 pipet 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 2.0 N 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 enymatic activity of the HRP. The resulting absorbance at 450 nm 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 kit utilizes two 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, the kit utilizes negative and positive controls. The negative control contains a concentration between the negative calibrator and the cut-off calibrator and the positive control contains a concentration of drug above the cut-off calibrator. These controls are used to assure the performance of the kit. To meet specifications the following validation criteria should be met:

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*.
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*.
3. The mean absorbance for the *negative calibrator* is greater than the mean absorbance for the *negative control*.
4. The mean absorbance for the *negative control* is greater than the mean absorbance for the *cut-off calibrator*.
5. The mean absorbance for the *cut-off calibrator* is greater than the mean absorbance for the *positive control*.

1.1.3.3 Assays in Use

Forensic Services utilizes the following assays:

Assay	*Calibrator*	*Urine Cut-off*	*Blood Cut-off*
Amphetamine Specific	d-Amphetamine	1000ng/mL	50ng/mL
Barbiturates	Secobarbital	200ng/mL	50ng/mL
Benzodiazepines	Blood: Nordiazepam Urine: Oxazepam	300ng/mL	50ng/mL
Cannabinoids	11-Nor-9-Carboxy-THC	50ng/mL	15 ng/mL
Cocaine Metabolite	Benzoylecgonine	300ng/mL	50ng/mL
Methamphetamine	Methamphetamine	1000ng/mL	50ng/mL
Opiates	Morphine	300ng/mL	50ng/mL

Refer to specific package inserts for complete details on each assay.

1.1.3.4 Protocols

1.1.3.4.1 Definition of Protocol

A protocol is a set of instructions that direct the PersonalLAB™ how to run a particular assay. Protocols exist for each of the seven 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.

1.1.3.4.2 Standard Protocols

Additional protocols may be created and used as necessary.

1.1.3.5 Profiles

The **PROFILE** is information the software uses to actually process the samples and generate results. The **PROFILE** should not be confused with a **PROTOCOL** (1.3.4), which includes the assay procedure steps, which are programmed when the instrument is initially set-up.

1.1.3.5.1 Definition of Profile

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). Assays are grouped as noted below.

Urine

Dilution	Assays in Profile
1:60*	Amphetamine, Barbiturates, Methamphetamine and Opiates
1:5	Benzodiazepines, Cannabinoids, and Cocaine Metabolite

* One part urine in a total of 60 parts.

Blood

Dilution	Assays in Profile
1:5	Cannabinoids
1:5	Amphetamine, Methamphetamine, Barbiturates and Benzodiazepines
1:5	Cocaine, Opiates, and Cannabinoids

1.1.3.5.2 Standard Profiles

Additional profiles may be created and used as necessary.

1.1.3.6 Interpretation of Results

1.1.3.6.1 Positive Result

A positive result for a sample is indicated by an absorbance less than or equal to the OraSure analyte Cut-off Calibrator. Depressed absorbances, which are significantly less than Negative Calibrator, can be interpreted as positives if the cross-reactivity for the analyte of interest is known to be low.

In addition, at the discretion of an analyst, confirmatory techniques may be applied to samples that exhibit depressed absorbances, 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. *Examples of cases where this exception could apply include infant testing and samples collected as the result of a drug recognition examination* (DRE).

1.1.3.6.2 Negative Result

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

1.2 OPERATION OF THE PersonalLAB

1.2.1 Preliminary Considerations

1.2.1.1 Supplies Required for Sample Dilution

1.2.1.1.1 Option one: Air-displacement pipettes and appropriate tips.

1.2.1.1.2 Option two: Repeater Pipette and appropriate tips.

1.2.1.1.3 Option three: Hamilton MicroLab® 500A series dilutor equipped with appropriate syringes.

1.2.1.2 Supplies Required for Testing

Supply	*Source*	*Comments*
5 mL disposable plastic culture tubes	BioChem	Dead volume = 200µL
75 mL plastic reservoirs	BioChem	Dead volume = 1.5mL
35 mL plastic reservoirs	BioChem	Dead volume = 1.0mL
5 mL plastic cups	BioChem	Dead volume = 200µL
Caps for cups	BioChem	
Disposable plastic pipette tips	BioChem	

1.2.1.3 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.
- Refer to OraSure Technologies Micro-plate Assay Package. Insert for instruction on the preparation of the cocaine assay enzyme conjugate.
- TMB substrate reagent (universal).
- 2N H_2SO_4 Stopping reagent (universal).

1.2.1.4 Processing of New Assay Kits

When a new assay kit is opened the following should be performed:

1.2.1.4.1 Check the expiration date of all components. The specification sheet will contain the expiration dates of serum calibrators and controls, micro-plates, enzyme conjugate, substrate and stopping reagent. The manufacturer in a technical bulletin as well as on individual bottles provides the expiration date of urine calibrators and controls.

1.2.1.4.2 Date and initial kit specification sheet and indicate whether the kit contains five or a single plate.

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

1.2.1.5 Quality Control (QC) Samples

1.2.1.5.1 Urine QC

The following QC samples must be included in each batch of urine specimens.

1.2.1.5.1.1 OraSure serum cut-off calibrator.
1.2.1.5.1.2 OraSure serum negative calibrator.
1.2.1.5.1.3 Negative control urine.
1.2.1.5.1.4 Positive control urine (BioRad, Utak, or equivalent).

1.2.1.5.2 Blood QC

The following QC samples must be included in each batch of blood specimens.

1.2.1.5.2.1 OraSure serum cut-off calibrator.
1.2.1.5.2.2 OraSure serum negative calibrator.
1.2.1.5.2.3 Negative control blood.
1.2.1.5.2.4 Positive control blood (In-house, Utak or equivalent).

1.2.2. General preparation for run.

Routine preparation for a run includes:

1.2.2.1 Fill wash bottles with distilled water.

1.2.2.2 Fill pipette tip tray with BioChem disposable tips.

1.2.2.3 Check printer paper supply.

1.2.3 General Rules of Operation

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

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

1.2.3.3 Do not push waste button while plate washing is taking place or vapor lock may occur.

1.2.3.4 Do not open lid when the *Operation Monitor* screen indicates that the lamp is warming. Opening the lid will result in the lamp continuing to warm indefinitely.

1.2.4 Blood calibrator and control preparation.

1.2.4.1 Calibrator Stock Standard Solutions

Drug standards (obtain as necessary from Cerilliant, Alltech, Sigma or equivalent vendor).

Stock (1.0mg/mL)	Potential Vendors
S-(+)-Amphetamine	Cerilliant A-008 1.0mg/mL
S-(+)Methamphetamine	Cerilliant M-020 1.0mg/mL
Benzoylecgonine	Cerilliant B-004 1.0mg/mL
Morphine	Cerilliant M-005 1.0mg/mL
(-)-11-nor-9-Carboxy-Δ9-THC	Cerilliant T-018 100µg/mL
Nordiazepam	Cerilliant N-905 1.0mg/mL
Secobarbital	Cerilliant S-002 1.0mg/mL

1.2.4.2 Calibrator Working Standard Solution*

Fill 10mL volumetric flask ~½ full with methanol. Add 50µL each of stock amphetamine, methamphetamine, benzoylecgonine, morphine, nordiazepam, and secobarbital. Add 150µL c-THC. Fill with methanol to 10mL line.

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

1.2.4.3 Quality Control Stock Standard Solutions

Drug standards (obtain as necessary from Cerilliant, Alltech, Sigma or equivalent vendor).

Stock (1.0mg/mL)	Potential Vendors
(+)Methamphetamine	Sigma M-5260 1.0mg/mL
D-Amphetamine	Sigma A-3278 1.0mg/mL
Benzoylecgonine	Sigma B-8900 1.0mg/mL
Morphine	Sigma M-9524 1.0mg/mL
11-nor-Δ9-THC-9-carboxylic acid	Sigma T-6893 50µg/mL
Secobarbital	Sigma S-4006 1.0mg/mL
Desmethyldiazepam	Sigma N-3162 1.0mg/mL

1.2.4.4 Quality Control Working Standard Solution *

Fill 10mL volumetric flask ½ full with methanol. Add 50µL each of amphetamine, methamphetamine, benzoylecgonine, morphine, secobarbital, and nordiazepam. Add 300µL C-THC. Fill with methanol to 10mL line.

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

Different vendors should be used to make up the Calibrator and Quality Control Working Solutions

1.2.4.5 Blood Calibrators

1.2.4.5.1 **Positive Calibrators**

Liquid Whole Blood (Utak 44600-WB (F) or equivalent) is spiked with calibrator working standard solution at 50%, 100% and 300% of cutoff. To 1mL of negative blood add working standard solution as indicated below.*

Desired % of cutoff	µL Working Standard Solution
50% cutoff	5
100% cutoff	10
300% cutoff	30

**Calibrators may be made using serial dilutions.*

1.2.4.5.2 **Negative Calibrator**

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

1.2.4.6 Blood Control

1.2.4.6.1 **Blood Quality Control (125% of Cutoff)**

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.

1.2.5 Sample collection and preparation.

1.2.5.1 Whole Blood Samples

1.2.5.1.1 Blood samples should be submitted in sodium fluoride (gray top) tubes or other tube types, as needed.

1.2.5.1.2 If particulates or clots are visible in the sample, homogenize with tissue grinder or clarify by centrifuging.

1.2.5.2 Urine Samples

1.2.5.2.1 Urine samples should be submitted in appropriate urine collection containers.

1.2.5.2.2 Samples with an unusually high turbidity should be centrifuged prior to analysis.

1.2.5.2.3 Urine samples should not contain the preservative sodium azide.

1.2.6 Off-Line Dilution of Samples

1.2.6.1 Option one: Calibrated air-displacement pipettes and appropriate tips.

1.2.6.2 Option two: Calibrated Repeater Pipette and appropriate tips.

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

1.2.6.4 Dilution of Samples

1.2.6.4.1 Prepare a 1 in 5 parts dilution in forensic diluent.

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

1.2.6.4.2 Prepare a 1 in 60 parts dilution in forensic diluent.

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

1.2.6.5 Appropriate Dilution for Each Assay

1.2.6.5.1 Urine

Dilution	Assays
1:60*	Amphetamine, Barbiturates, Methamphetamine and Opiates
1:5	Benzodiazepines, Cannabinoids, and Cocaine Metabolite

* One part urine in a total of 60 parts.

1.2.6.5.2 Blood

Dilution	Assays
1:5	Cannabinoids
1:5	Amphetamine, Methamphetamine, Barbiturates and Benzodiazepines
1:5	Cocaine, Opiates, and Cannabinoids

1.2.6.6 Dilution of Calibrators and Controls

1.2.6.6.1 Dilution of calibrators and controls should be performed as noted under sections 1.2.6.4 and 1.2.6.5.

1.2.7 Initial Start-up / Session Preparation

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

1.2.7.2 Prepare samples for analysis. Dilute as indicated under sections 1.2.6.4 and 1.2.6.5.

1.2.7.3 Turn on computer.

1.2.7.4 Click on *Plab wb* icon (wb = workbench). Instrument will print-out *BIOCHEM ImmunoSystems, INC.* and the date.

1.2.7.5 From the *WorkBench – v1.1a* screen, log-on by clicking on the icon. Enter user name, press **Tab** and then enter password.

1.2.7.6 Daily maintenance can either be proceeded with at this time or at *Step* 1.2.7.26.

1.2.7.6.1 Click on icon. From the *open* screen select ⊙**Session** {***Figure 1***} from list.
Click **OK**

1.2.7.6.2 From *open session* screen, select *Start-up Maintenance.tpl* from "Template List" {Figure 2}. **Double click.**

1.2.7.6.3 Template will show up in lower "file list" box.
Double click in box on selection **or** highlight selection and click **OK**

1.2.7.6.4 Click on *Start Session* icon (far right /red arrow).

1.2.7.6.5 When *Profile –Vial Locations for Controls or Standard and Reagents* view comes up {Figure 5}, click Continue.

1.2.7.6.6 Screen will indicate *Waiting for Initialization.*

1.2.7.6.7 *Start-up* folder tab screen comes up.

1.2.7.6.8 To select *Self Test*, Press Start. Instrument will check motors and voltages for acceptability.
Display will inquire, *Print Self Test* report? Press Yes.

1.2.7.6.9 *Fill Syringes*
After priming, screen will inquire *Continue?*
Indicate Yes if bubbles are observed.
Press No, when bubbles are not longer present.

1.2.7.6.10 *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

1.2.7.6.11 ***Note: Overfilling lung can result in instrumental problems that may require a service call to remedy. The level sensing ability of the lung may be damaged.***

1.2.7.6.12 Screen will now instruct operator to *close cover to continue operation.*
Click **OK** .

1.2.7.6.13 *Buffer (Tank) 1 Prime* at least twice.
Watch tubing lines for bubbles.

1.2.7.6.14 *Buffer (Tank) 2 Prime* at least twice.

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

1.2.7.7 Click on Wrench icon (far left) to reset plastic tip counting.

1.2.7.7.1 Click on Reset

1.2.7.7.2 Click on **OK**

1.2.7.7.3 When daily maintenance is complete click ☒.
Display will inquire *End-of-work has not been executed – Continue exiting?* Select Yes.

1.2.7.8 Click on 🗁 icon. From the *open* screen select ⊙**SESSION** {***Figure 1***} from list. Click **OK**

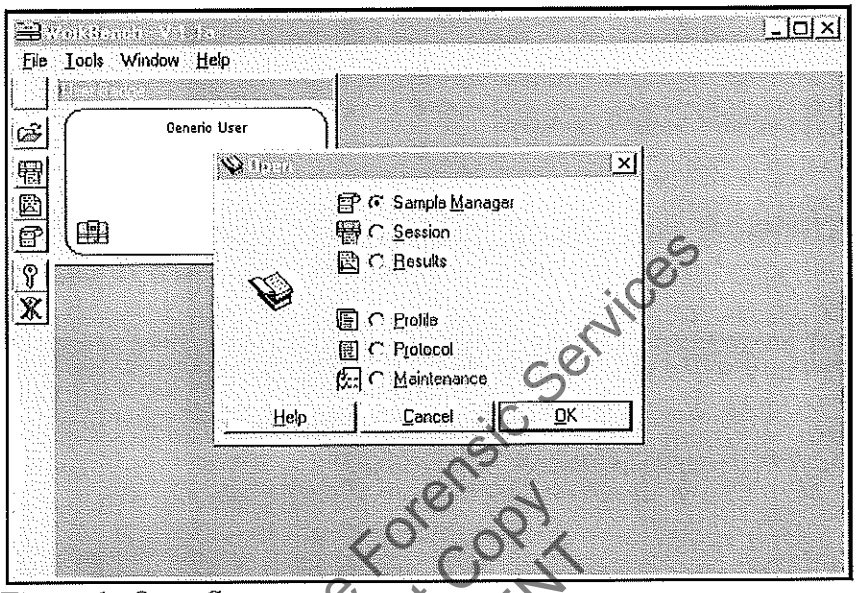

Figure 1: Open Screen

1.2.7.9 From *open session* screen, select appropriate template from "Template List" {Figure 2}.

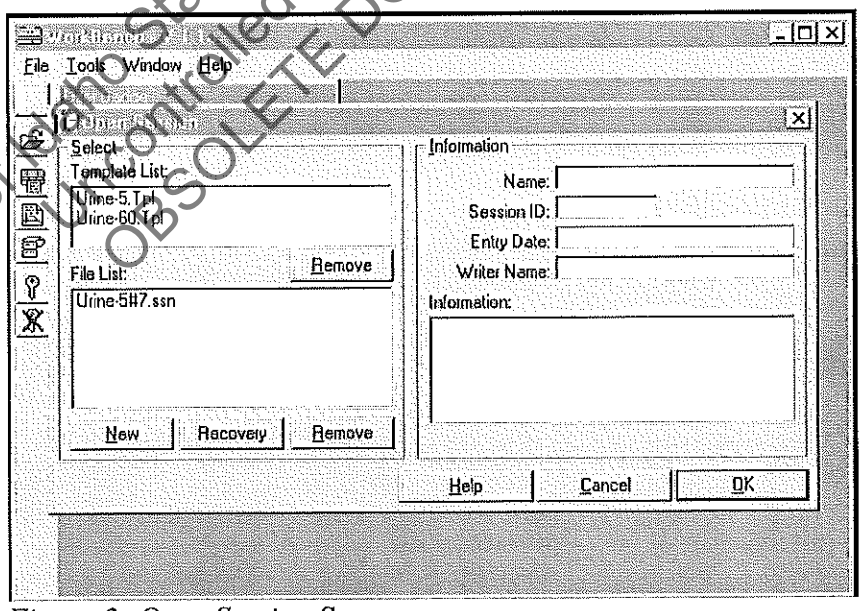

Figure 2: Open Session Screen

1.2.7.9.1 Select appropriate template.

1.2.7.9.2 Template will show up in lower "file list" box. **Double click** in box on selection or click **OK**

1.2.7.10 *Session* screen will now come up {Figure 3}. Click on test tube icon to bring up *Sample Programming Screen* {Figure 4}.

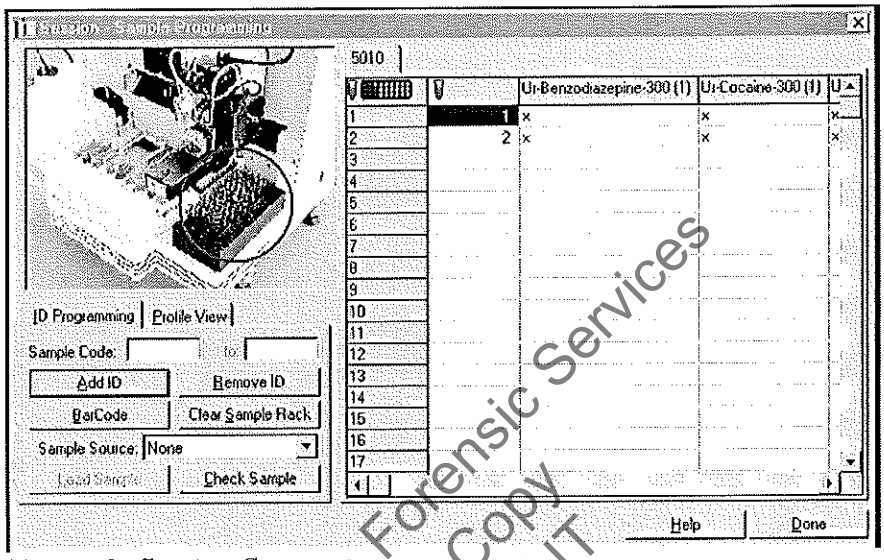

Figure 3: Session Screen

1.2.7.11 With *Session Sample Programming* screen displayed, click on sample rack of choice {Figure 4}.

1.2.7.11.1 Select appropriate rack.

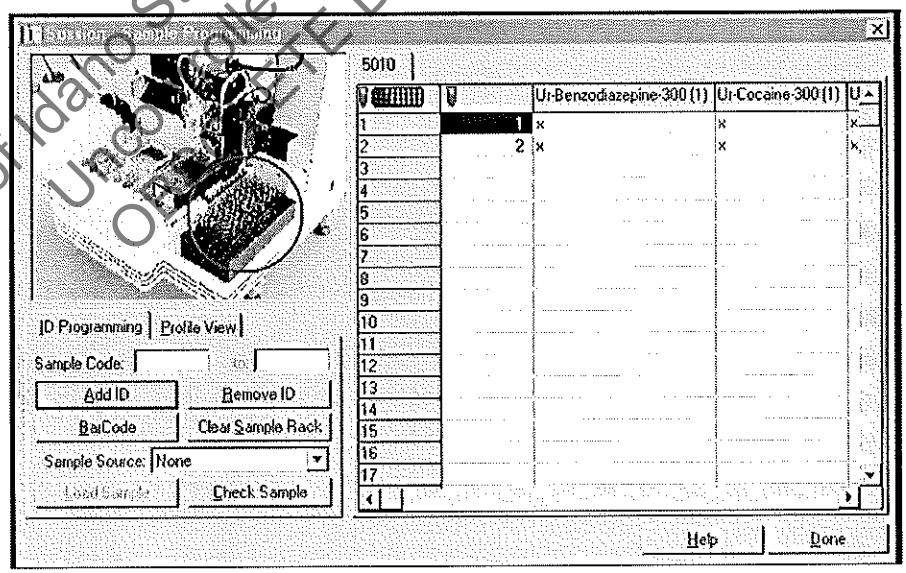

Figure 4: Sample Programming Screen

1.2.7.12 To clear previous programming, click on Clear Sample Rack.

1.2.7.13 If re-running samples DO NOT PRESS CLEAR SAMPLE RACK.

1.2.7.14 On *Session - Sample Programming* screen enter "Sample Codes" in the box on the left portion of the screen. Number will appear on the right portion of the screen after Enter is pressed. Input either the laboratory number of the specimen or enter the source information for positive and negative controls.

1.2.7.15 Double click left on rack icon.

1.2.7.15.1 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 assays.

-or-

Highlight desired sample boxes under assay and double click right mouse button while cursor is in highlighted area.

1.2.7.16 Click DONE

1.2.7.17 *Session – Protocol Position"* page then comes up {Figure 5}.

1.2.7.17.1 This view of the plate racks illustrates the number and position of the individual strips, which are necessary for each individual assay.

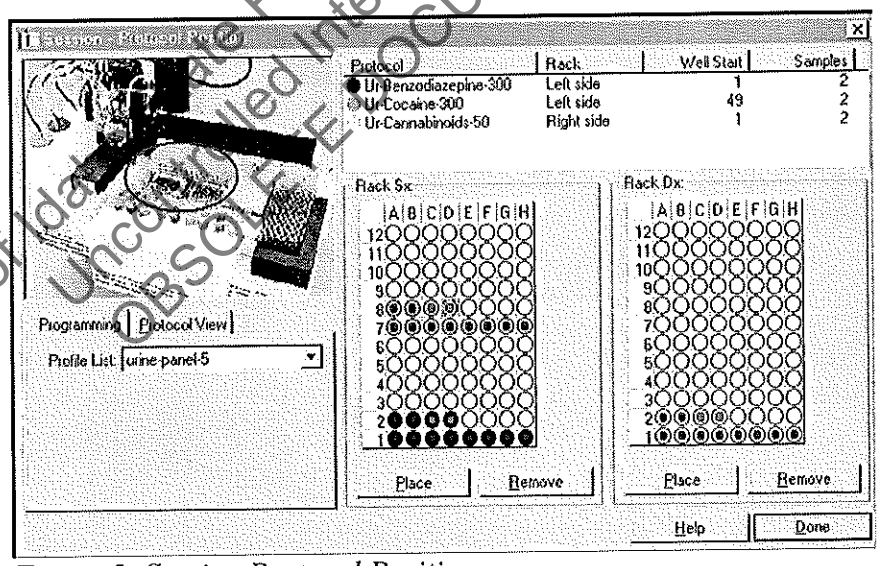

Figure 5: Session Protocol Position

1.2.7.18 Load plate racks with appropriate strips.

1.2.7.18.1 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.

1.2.7.19 Click DONE

1.2.7.20 Save *Session* by clicking on save or on 🖫 (save session).

1.2.7.21 To print the sample load list

1.2.7.21.1 Click on *File View* icon from the tool bar (3rd from left). The *View File* page will come up.

1.2.7.21.2 Click on Document icon from tool bar (2nd icon from left).

1.2.7.21.3 Click on Print icon from the toolbar.

1.2.7.21.4 Click on ☒ to go back to *Session* page.

1.2.7.22 Load sample rack.

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

1.2.7.23.1 *Profile –Vial Locations for Controls or Standard and Reagents* view comes up {Figure 6}.

1.2.7.24 Load cups and reagent reservoirs onto platform.

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

1.2.7.24.2 Place appropriately diluted amount of each control (negative and positive) and calibrator (negative and cutoff) into the 5mL cups. Refer to sections 1.2.5.4 and 1.2.5.5 for appropriate dilutions. Place the cup at its designated numbered location.

1.2.7.24.3 Place serum negative calibrator and cut-off calibrator in 5mL cups at appropriate locations.

1.2.7.24.4 After loading is complete, hit Continue.

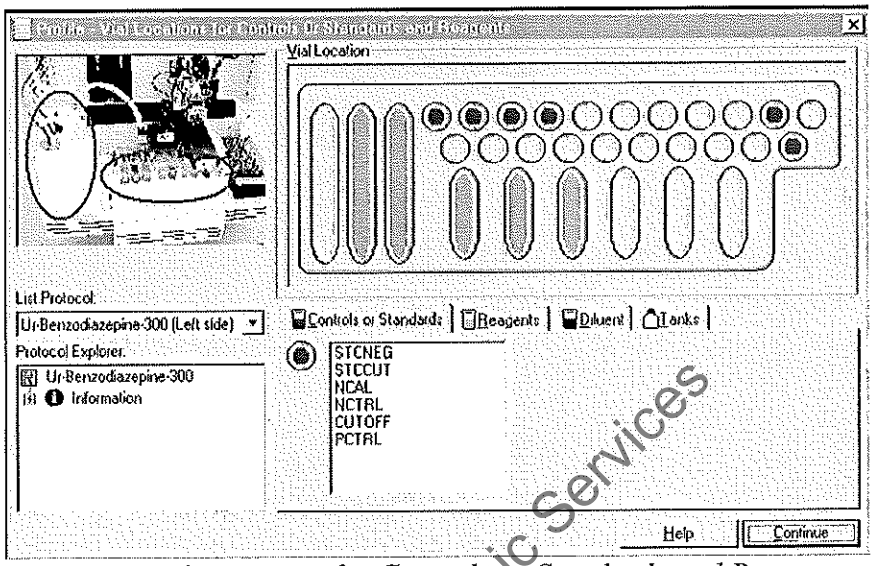

Figure 6: Vial Locations for Controls or Standards and Reagents

1.2.7.25 If maintenance is not already complete, turn instrument on.

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

1.2.7.26 DAILY MAINTENANCE

If not performed initially, it should be completed at this time. If daily maintenance has been performed proceed to *Step 1.2.7.28.*

From *Station: 0 Processor V 1.1a* screen, click on *START-UP* folder tab to perform daily maintenance {Figure 7}. Click on appropriate boxes to:

1.2.7.26.1 Run *Self-Test.* Click on *Start.*

1.2.7.26.2 Display will inquire, *Print Self-Test* report? Press Yes.

1.2.7.26.3 *Fill Syringes*
After priming, screen will inquire *Continue?*
Indicate Yes if bubbles are observed.
Press No, when bubbles are no longer present.

1.2.7.26.4 *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 .

1.2.7.26.5 Buffer (Tank) 1 Prime.
Watch tubing lines for bubbles.

1.2.7.26.6 Buffer (Tank) 2 Prime.
Watch tubing lines for bubbles.

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

1.2.7.27 Click on Wrench icon (far left) to reset plastic tip counting.

1.2.7.27.1 Click on Reset

1.2.7.27.2 Click on **OK**

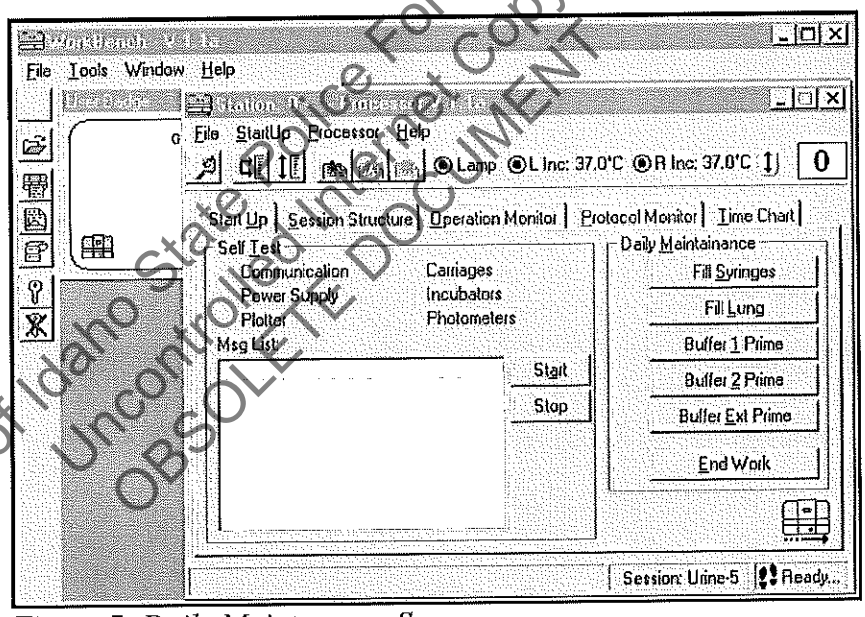

Figure 7: Daily Maintenance Screen

1.2.7.28 From *Station: 0 Processor V 1/1a* page, click on *Operation Monitor* folder tab.

1.2.7.28.1 Click on EXE icon (4th from left).

1.2.7.29 In preparation for the run:

- Instrument will remind you about rack placement.
  - Operator can *retry* if sample rack is not in place.
- Screen will indicate *Waiting for Lamp Warm-up.*
- Instrument will check strip/racks in carriages.

1.2.7.30 Operation monitor will indicate the following tabs:

Time	*Task*	*Profile*	*Protocol*	*Rack*

1.2.7.31 To monitor run, click on profile tab.

1.2.7.32 When run is complete *Processor Screen* will indicate *Session terminated.* If end-of -work (refer to section 1.2.10) is not to be pursued at this point, click on **OK** followed by ☒.

1.2.8 Obtaining Results

1.2.8.1 From *WorkBench* screen, click on Paper (Results) icon (four down from top)

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

1.2.8.1.2 Click OK

1.2.8.1.3 Select Report icon (far right).

1.2.8.1.4 Refertation (data reduction) will commence.

1.2.8.1.5 When processing of data is complete click on Print icon.

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

1.2.8.2 To access results not immediately following a run:

1.2.8.2.1 Click on Open Folder icon (folder with arrow).

1.2.8.2.2 Click on Open results.

1.2.8.2.3 Highlight Session ID, Name or Entry Date.

1.2.8.2.4 Click OK

1.2.8.2.5 Select *New* results or *Database* results (old/ previous).

1.2.8.2.6 Highlight either selection, click OK

1.2.9 Re-Reading of a Plate

1.2.9.1 Click on icon. From the *open* screen select ⊙**SESSION** {***Figure 1***} from list. Click **OK**

1.2.9.2 From *open session* screen, select appropriate template from "Template List" {Figure 2}.

1.2.9.2.1 Select previously run template.

1.2.9.2.2 **Double click.**

1.2.9.2.3 Template will show up in lower "file list" box.

1.2.9.2.4 Double click in box on selection or click **OK**

1.2.9.3 *Session* screen comes up. Click on test tube icon *(sample programming).*

1.2.9.4 Click on sample rack that samples were run in.

1.2.8.4.1 Select appropriate rack.

1.2.9.5 { } will indicate "done".

1.2.9.6 **Do not click on *Clear Sample Rack*.**

1.2.9.7 Highlight samples run. Screen will turn blue. Double click RIGHT in highlighted area. Click DONE.

1.2.9.8 *Session – Protocol Position"* page then comes up {Figure 4}. Click DONE

1.2.9.9 Save *Session* by clicking on save or on 💾 (save session).

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

1.2.8.10.1 *Profile –Vial Locations for Controls or Standard and Reagents* view comes up {Figure 5}. Hit Continue.

1.2.9.11 From *Station: 0 Processor V 1.1a* page, select *Time Chart* folder tab.

1.2.9.12 On *Time Chart,* scroll down to *Reading* step. Highlight from *Reading* to *End.*

1.2.9.13 Click on Steps Select icon (second from left)

1.2.9.14 Click on EXE icon (fourth from left).

1.2.9.14.1 *Reading* Step comes up. Screen will indicate Waiting for Lamp Warm-up. Lamp will warm up for each re-read.

1.2.9.15 When re-read is complete, display will indicate *Session Terminated.* Click **OK**.

1.2.9.16 Obtain results as described in *section 1.2.7.*

1.2.10 End-of-day Clean up

1.2.10.1 Return conjugates, stop and diluent reservoirs to refrigerator.

1.2.10.2 Dispose of used calibrator, controls, micro-plates and samples into appropriate biohazard container.

1.2.10.3 Fill plastic tip tray.

1.2.10.4 For *End-of-Work* routine, select START-UP tab.
1.2.10.4.1 Click on End of Work.

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

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

1.2.10.7 Click on ☒ to close *WorkBench* window.

1.2.10.8 Record Daily Maintenance in PersonalLAB QC binder.

1.3 PERIODIC MAINTENANCE SCHEDULES

1.3.1 Weekly and Monthly Maintenance

1.3.1.1 Refer to the Maintenance section of the PersonalLAB™ notebook for maintenance schedules and tasks to be performed on a weekly and monthly basis.

1.3.1.2 *Start-up Maintenance Template* can be used to access priming functions.

1.6 QUALITY CONTROL

1.6.1 Assay Displacement Calculation

1.6.1.1 Percent displacement should be calculated based upon the values obtained from the OraSure serum cut-off calibrator and serum negative calibrator.

1.6.1.2 Calculate displacement as follows:

$$\%\text{Displacement to Cutoff} = \frac{A_{450}\text{ Value (Serum Negative Calibrator)} - A_{450}\text{ Value (Serum Cutoff Calibrator)}}{A_{450}\text{ Value (Serum Negative Calibrator)}} \times 100$$

1.6.1.3 Compare the calculated percent displacement with the acceptable range for the displacement provided on the specification sheet for the particular lot of each assay.

1.6.1.3.1 Percent displacement values should fall within ±10% of the package insert assay range.

1.6.1.3.2 If the percent displacement values do not fall within ±10% of the assay range, the particular assay should be repeated.

1.6.1.4 Record % displacement on original assay print-out.

1.7 DISTRIBUTION OF ASSAY INFORMATION

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

1.7.2 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.

1.7.3 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.

1.8 REFERENCES

1.8.1 Butler, J.E. **Enzyme-Linked Immunosorbent Assay**. pp. 759-803 *In*: "Immunochemistry". Van Oss, C.J.; van Regenmortel, M.H.V., eds., Marcel Dekker, inc., New York, NY: 1994.

1.8.2 PersonalLAB™ User's Manual, 080040-001 REV.01, 1998.

1.8.3 OraSure Technologies PersonalLAB™ Training Guide.

1.8.4 OraSure Technologies Package Inserts for Serum Microplate EIA.

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
0	04-24-00	Original Issue
1	04-24-02	Updated and made STC name change corrections
2	09-13-02	Clarification of distribution of assay information (1.7.1)
3	01-03-02 JMY 01-03-03	Further clarification of distribution of assay information (1.7.1)

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

History Page

Toxicology Training Manual –
Section Two: Blood and Urine Ethanol and Other Volatiles

Revision #	Issue Date	History
0	05-30-00	Original Issue
1	12-16-02	Updated to comply with Quality Manual

Approval

Technical Leader: _____ **Date:** 12-16-02

Issuance

QC Manager: _____ **Date:** Jan. 2, 2002
Richard D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Two

Blood and Urine Ethanol and Other Volatiles

2.1 Training Objectives

This section of the toxicology training manual is designed as a guide to provide a forensic chemist with sufficient background to not only gain proficiency with blood alcohol analysis, but also to acquire an understanding of the variety of situations surrounding the collection of biological specimens for alcohol analysis. This manual deals with sample preparation for analysis with a gas chromatograph equipped with a headspace analyzer. For this analysis to stand-up in court, good analytical practices should be adhered to. These practices include rigorous acceptance criteria for data as outlined in the SOP for analysis. In order to address questions in court, the analyst must possess a basic knowledge of the pharmacology of ethanol and related compounds.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.2 Evidence Handling

2.2.1 Specimens Submitted for Alcohol and/or Volatiles Analysis
The trainee should display an understanding of the procedures followed for the intake of specimens and subsequent specimen handling considerations.

2.2.2 The trainee should describe what IDAPA 11.03.01 mandates as the proper way to collect a blood and a urine sample for forensic ethanol analysis.

2.2.3 Familiarization with the Alcohol and/or Volatiles Analysis Program
The trainee should display an over-all understanding of the manner in which specimens are processed, the agencies served, the programs involved and casework expectations.

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Trainer

2.2.4 References

1. ASTM E1459-92, *Standard Guide for Physical Evidence Labeling and Related Documentation.*

2. Kippenberger, D.J. and Selavka, C.M. *Training in Specimen Handling*. pp. 33-54, *in:* California Association of Toxicologists (CAT) Manual for Analytical Toxicology. 1994.

3. IDAPA 11, Title 03, Chapter 01: Idaho State Forensic Laboratory Rules Governing Alcohol Testing.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.3 Solution Preparation

2.3.1 The trainee should demonstrate the ability to prepare solutions required in the analysis of alcohol and other volatiles.

2.3.2 The trainee should be acquainted with the nomenclature and calculations involved in the determination of weight percent and volume percent solutions.

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2.3.3 References

1. College Chemistry Text, chapter(s) discussing the properties of solutions.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.4 Gas Chromatography (GC) Theory and Practice

2.4.1 The trainee should have comprehensive background in regards to the principles of GC.

2.4.2 The trainee should provide a brief explanation of GC in terms understandable to a layperson.

2.4.3 The trainee should demonstrate their ability to operate and maintain a GC-Flame Ionization Detector (FID). This includes an understanding of the system's software, inlet and detector maintenance, column installation, and troubleshooting techniques.

2.4.4 Describe the influence carrier gas flow has on the efficiency of a GC-FID.

2.4.5 Define the following terms as they relate to GC.

2.4.5.1 *Resolution*
2.4.5.2 *Area Under the Curve*
2.4.5.3 *HETP*

2.4.6 Discuss which GC parameters affect resolution. Describe how to approach a lack of resolution.

2.4.7 Discuss how to alleviate peak tailing.

2.4.8 The trainee should possess an understanding of the principles and application of quantitative analysis.

2.4.9 Describe the major advantages of using an internal standard method.

2.4.10 State how blood and urine alcohol results are to be reported in terms of units employed.

Date of Completion _____ Trainee _____

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2.4.11 References

1. Stafford, D.T. *Chromatography.* pp. 93-101, 103-114, *in:* Principles of Forensic Toxicology, edited by Barry Levin, , AACC, 1999.

2. Levine, B. *Alcohol.* pp. 170-184, *in:* Principles of Forensic Toxicology, edited by Barry Levin, AACC, 1999.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.5 Headspace Analysis Theory and Practice

2.5.1 Trainee should possess a working knowledge of the theory and practice of headspace analysis.

2.5.2 Describe how *the proportionality* known as *Henry's Law*, is utilized in headspace analysis.

2.5.3 The trainee should demonstrate their ability to operate and maintain a Headspace Analyzer. This includes an understanding of the system's software, maintenance, and troubleshooting techniques.

2.5.4 The trainee should be acquainted with how the headspace method parameters such as the GC cycle time, thermostating time, pressurization time, etc., should be optimized.

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2.5.5 References

1. Stafford, D.T. *Chromatography.* pp. 93-101, 103-114, *in:* Principles of Forensic Toxicology, edited by Barry Levin, AACC, 1999.

2. Saker, E.G. *Screening and Quantitation by Headspace Technique of Some of the Vapors Most Commonly Found in Forensic Toxicology.* pp. 1-33, *in*: Current Approaches in Forensic Toxicology, Chapter 11, SOFT Meeting, 1994.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.6 Basic Alcohol Pharmacology

2.6.1 The trainee should have a working knowledge of the pharmacology of alcohol. This should include an understanding of the factors affecting its absorption, distribution and elimination.

2.6.2 Describe the situation when the alcohol content of arterial blood exceeds that of venous blood.

2.6.3 The trainee should be familiar with the metabolism of ethanol and other commonly ingested alcohol in regards to how alcohol metabolism relates to their toxicity.

2.6.4 The trainee should have an understanding of the effects of alcohol on the human body. This should include how it contributes to mortality and impairment observed in DUI cases.

2.6.5 The trainee should be comfortable with how field sobriety tests are administered.

2.6.6 The trainee should be acquainted with how ethanol use affects an individual's performance on field sobriety tests.

_____ _____
Date of Completion Trainee

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Trainer

2.6.7. References

1. Levine, B. *Alcohol.* pp. 170-184, *in:* Principles of Forensic Toxicology, edited by Barry Levin, AACC, 1999.

2. Kunsman, G.W. *Human Performance Testing.* pp. 170-184, *in:* Principles of Forensic Toxicology, edited by Barry Levin, AACC, 1999.

3. Caplan, Y.H. *The Determination of Alcohol in Blood and Breath.* pp. 594-648, *in:* Forensic Science Handbook, edited by Richard Saferstein, New Jersey:Prentice-Hall, 1982.

4. Julien, R.M. *Central Nervous System Depressants: Alcohol and the Inhalants of Abuse*. pp. 64-92, *in:* Primer of Drug Action, New York:Freeman, 1998.

5. Perrine, D.M. *Depressants: Alcohol, Benzodiazepines, Barbiturates*, pp. 113-129, *in:* The Chemistry of Mind-Altering Drugs, ACS, Washington, DC, 1996.

6. Hobbs, W.R., Rall, T.W. and Verdoorn, T.A. *Drugs Acting on the Central Nervous System - Hypnotics and Sedatives; Ethanol.* pp. 361, 386-393, *in:* Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, 1996.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.7 Other Devices

2.7.1 Artel Pipette Calibrator

2.7.1.1 The trainee should have a working knowledge of how to prepare the PCS 2™ Pipette Calibration System to perform pipette calibration.

2.7.1.2 Describe the operating principle of the PCS 2™ Pipette Calibration System.

2.7.1.3 Explain the routine maintenance performed on the PCS 2™ Pipette Calibration System.

2.7.1.4 List ten practices that will improve pipetting technique.

Date of Completion _____ Trainee _____

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2.7.1.5 References

1. PCS 2™ Pipette Calibration System Procedure Guide.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.7 Other Devices

2.7.2 Hamilton MICROLAB® Dilutor

2.7.2.1 The trainee have a working knowledge of the Hamilton MICROLAB® dilutor.

2.7.2.2 Describe the routine maintenance performed on the Hamilton MICROLAB® dilutor.

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2.7.2.3 References

1. Hamilton MICROLAB® User's Manual.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.8 Generation and Interpretation of Data

2.8.1 The trainee possesses a thorough understanding of the Toxicology Program Methods Manual section 4.1, *Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles by Dual Column Headspace Gas Chromatography.*

2.8.2 The trainee possesses a thorough understanding of the requirements that must be met for the qualitative identification of volatiles and the quantitation identification of alcohol in blood, urine and vitreous humor.

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2.8.3. References

1. Toxicology Program Methods Manual, section 4.1, *Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles by Dual Column Headspace Gas Chromatography.*

2. IDAPA 11, Title 03, Chapter 01: Idaho State Forensic Laboratory Rules Governing Alcohol Testing.

Section Two

Blood and Urine Ethanol and Other Volatiles

2.9 Casefile Preparation

2.9.1 The trainee should be aware of the items that are required to be included in an alcohol/other volatiles analysis casefile.

2.9.2 The trainee should be familiar with the worksheets used to record the results of analysis.

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Trainer