Idaho State Police
Forensic Services

Toxicology Methods Manual

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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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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 ℃.

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 ℃.

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**

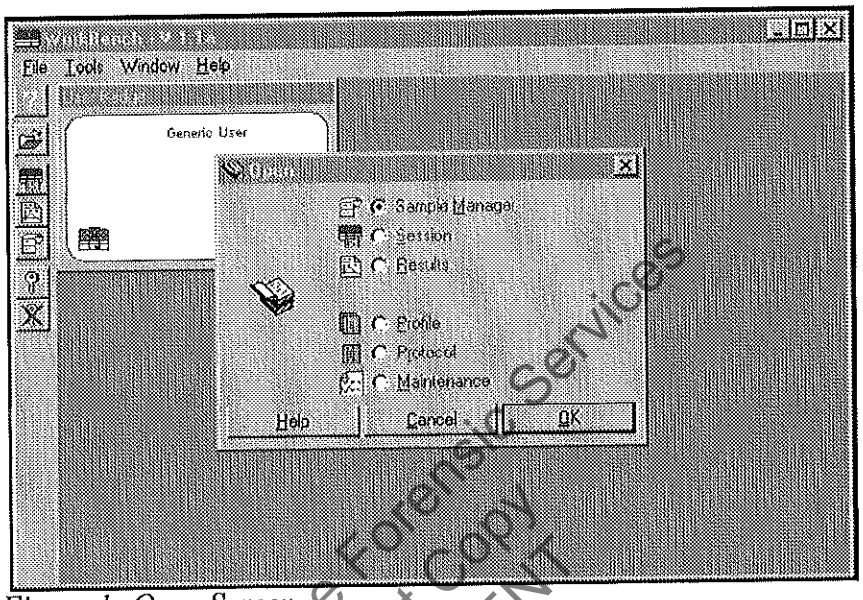


Figure 1: Open Screen

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

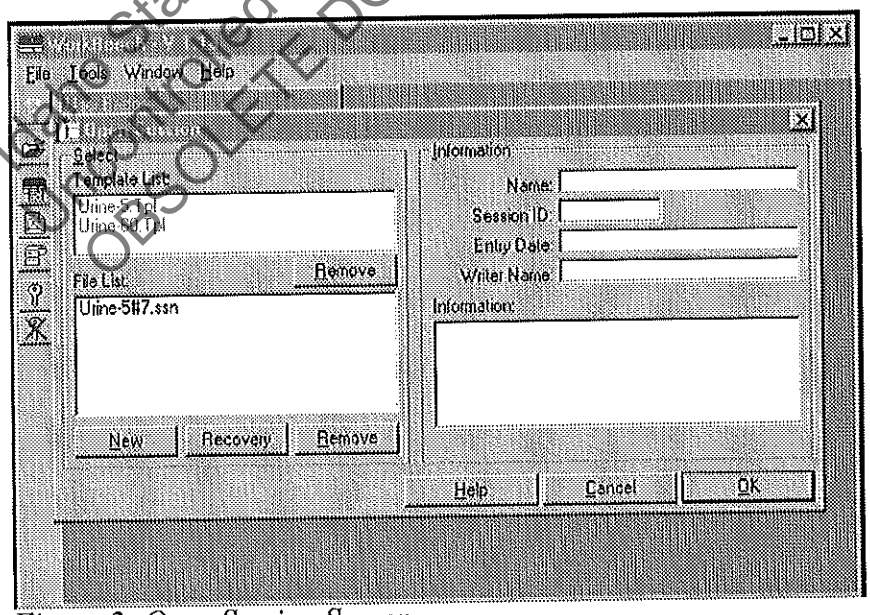


Figure 2: Open Session Screen

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}.

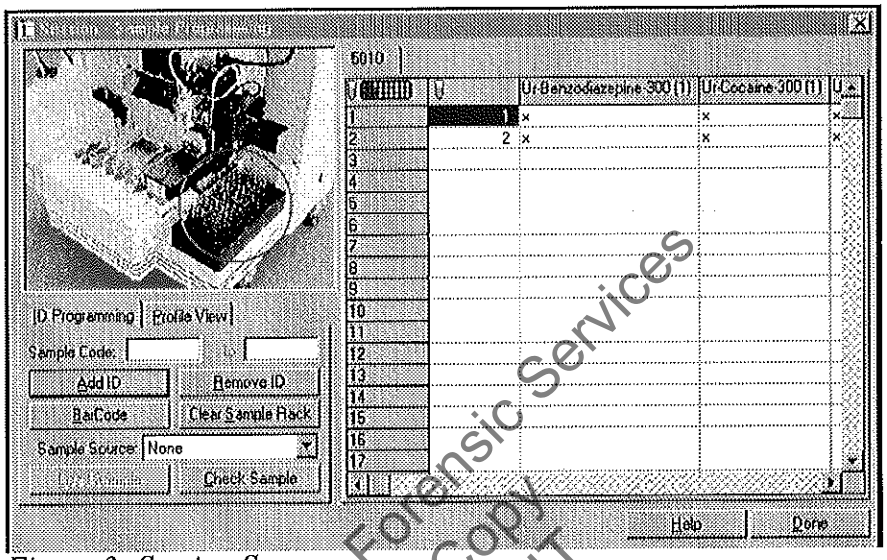


Figure 3: Session Screen

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.

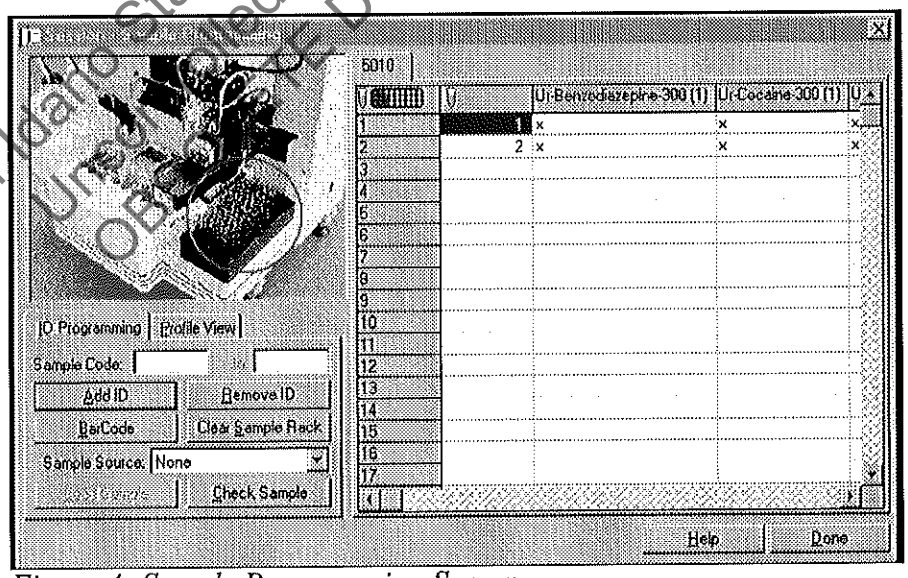


Figure 4: Sample Programming Screen

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.

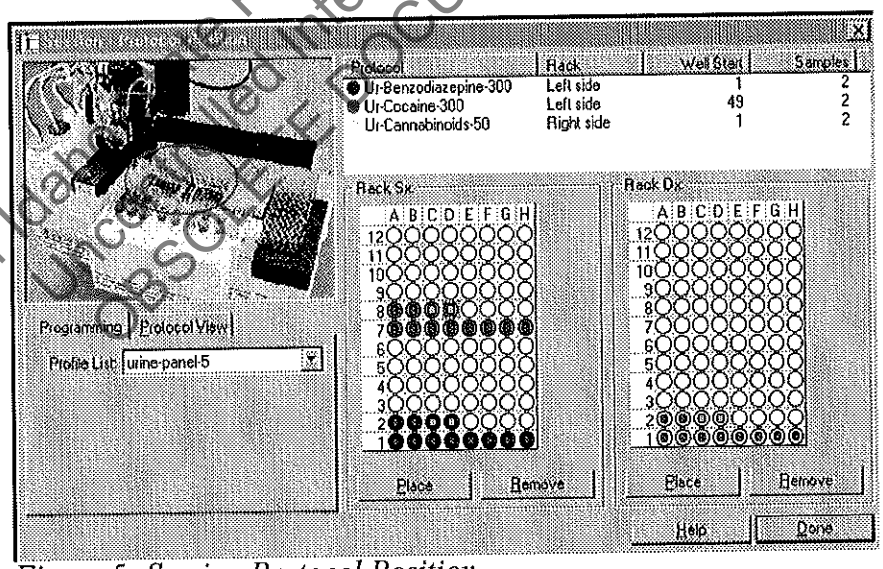


Figure 5: Session Protocol Position

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.

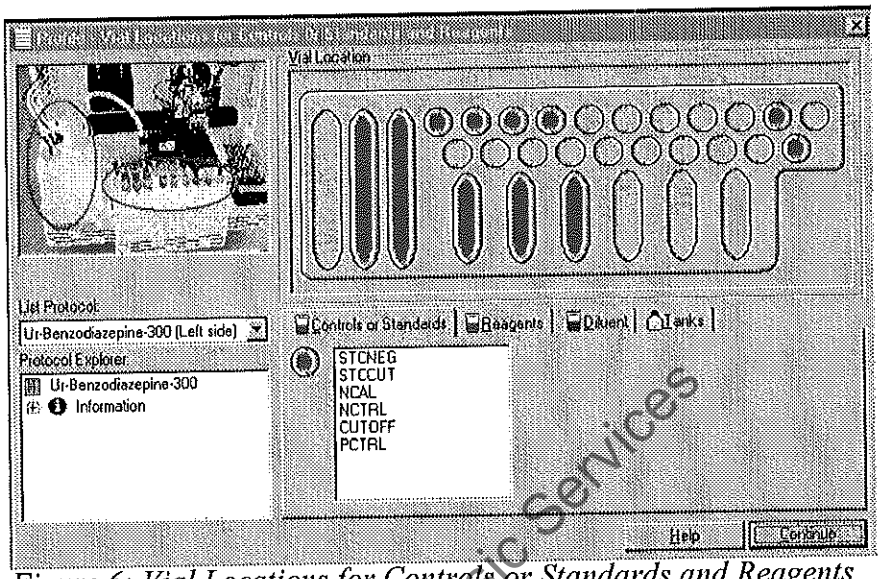


Figure 6: Vial Locations for Controls or Standards and Reagents

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**

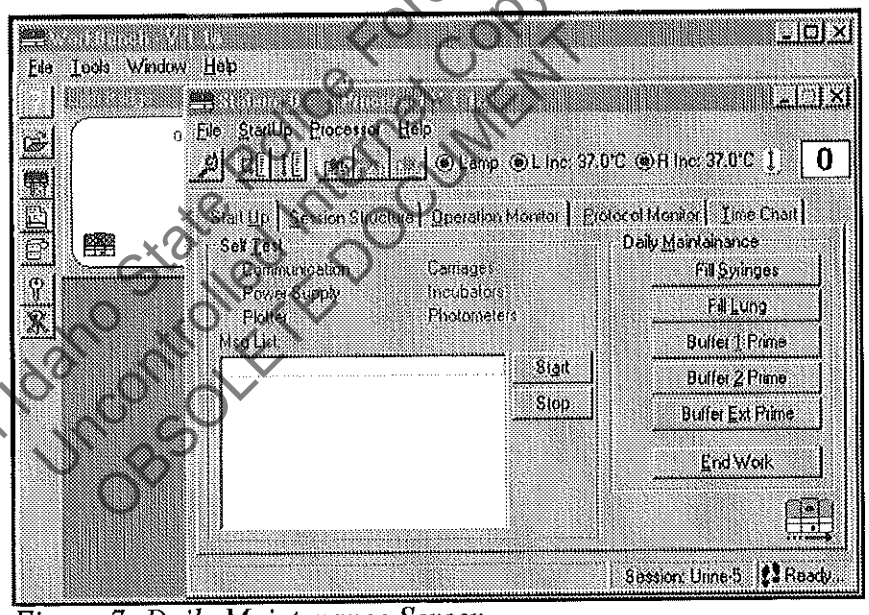


Figure 7: Daily Maintenance Screen

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 Calculation of Displacement
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 ±5% of the package insert assay range.

1.6.1.3.2 If the percent displacement values do not fall within ±5% 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 Printouts

1.7.1.1 Original data from each assay should be maintained in a binder on an annual basis.

1.7.1.2 A cover sheet containing the date of the run and the lot number for each assay should be included with original data.

1.7.1.3 Assay results are recorded on data page of toxicology analysis form.

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 Four
Analysis of Alcohol and Common Volatile Solvents

4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles by Dual Column Headspace Gas Chromatography

4.1.1 BACKGROUND

Fermented beverages such as beer and wine have been known and used by humans since prehistoric times.[6] Ethanol abuse is often manifest in driving under the influence (DUI) problems, which is a worldwide concern. The National Highway Traffic Safety Administration (NHTSA) estimates that alcohol was involved in 41% of fatal automobile crashes and 7% of all crashes in 1995.[2] Chronic alcoholism also contributes to ethanol related deaths. Ethanol consumed on a regular basis can lead to the development of alcoholic hepatitis which can progress into cirrhosis, liver failure, and death.[2,6,7] Chronic excessive ingestion of ethanol is directly associated with serious neurologic and mental disorders such as brain damage, memory loss, sleep disturbances and psychoses.[7] Alcohol is also involved in a high percentage of domestic disputes many of which result in injury and/or death.

Notwithstanding the public perception that ethanol is stimulatory, ethanol is classified as a *Central Nervous System Depressant.* Ethanol is a psychoactive drug that is similar in most respects to sedative-hypnotic compounds.[4] The first mental processes to be affected are those that depend on training and previous experience.[7] The individual's memory, concentration, and insight are dulled and subsequently lost. The person may become overly confident and exhibit uncontrolled mood swings and/or emotional outbursts.[7] The effects of ethanol and other central nervous system depressants are additive, resulting in more sedation and greater impairment of driving ability.[4]

Ethanol is rapidly and completely absorbed from the stomach, small intestine and colon. The mechanism of absorption is a simple diffusion process, that is, alcohol moving from a region of higher to a region of lower concentration.[2,4,6] Alcohol is soluble in both water and fat, a property that facilitates its diffusion through biological membranes.[4] The major amount of absorption takes place in the small intestine due to its large surface area, good blood supply and thin walled membrane. The time from the last drink to peak concentrations can range between 30 and 90 minutes, depending upon the individual's stomach contents.[4,7] Alcohol absorption is slowed by the presence of food in the stomach. The time period required for gastric emptying is a prime factor that contributes to

the wide variety of absorption rates of ingested ethanol observed in different individuals and under different conditions.[2,7] Hence, the extent of absorption in the stomach and small intestine is a function of the amount of ethanol at that site, the vascularity of the site and the surface area in contract with the blood supply.[2] Other factors that affect the absorption of ethanol include the type of beverage, the alcohol content and any disease state that affects normal gastric function.[2]

Upon absorption, ethanol is distributed to all the water containing regions of the body. Within the blood there can be significant differences between arterial and venous blood depending upon the absorption status of the individual.[2] In the absorptive phase, the arterial blood ethanol concentration exceeds the venous blood ethanol concentration. Analysis of venous blood therefore, underestimates the brain alcohol concentration of the individual at this point. When absorption is complete there is little difference in ethanol concentration between and arterial and venous blood.[2]

90 to 98 percent of ethanol is completely oxidized in the liver by reacting with the cofactor nicotinamide adenine dinucleotide (NAD) facilitated by alcohol dehydrogenase to produce acetaldehyde. Acetaldehyde is then acted upon by aldehyde dehydrogenase to form acetic acid which goes onto form carbon dioxide and water (figure 1). The amount of ethanol oxidized per unit time is roughly proportional to body weight and probably to liver weight. The remaining (unoxidized) alcohol is excreted unchanged in urine, expired air, saliva and sweat. The average elimination rate of ethanol is 0.015 g/dL/hour from men and 0.018 g/dL/hour for women.[2] In addition to gender, chronic abuse, ethanol use combined with prescription drugs and certain genetic factors can also influence the elimination rate.[2,6,7]

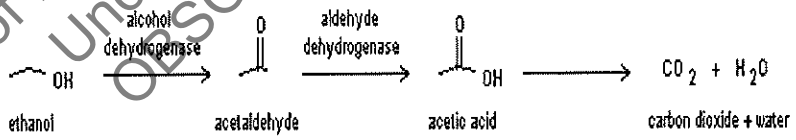


Figure 1. Metabolism of Ethanol.

Methanol (wood alcohol) causes relatively little intoxication compared to ethanol.[2,6] Its harmful affects are due to the direct result of its metabolism to formaldehyde (embalming fluid) and subsequently to formic acid. These metabolites lead to the destruction of neural cells, particularly the optic nerve, which can result in blindness.[2,6]

4.1.2 PRINCIPLE

This method describes the analysis of aqueous samples for the presence of volatile compounds including methanol, ethanol,

acetaldehyde, acetone, isopropanol and related compounds, via a headspace sampling gas chromatographic method. Samples, controls and standards are sealed into vials that contain an aqueous 1-propanol internal standard solution and heated by the headspace analyzer. As described in Henry's Law, in a closed container at a given temperature, a direct (proportional) relationship exists between the amount of a volatile substance dissolved in a liquid and the amount of the volatile substance in the headspace vapor above the solution. An aliquot of the vapor is injected into a gas chromatograph (GC) in a dual column configuration. The GC serves to separate out the components of the solution as a function of their chemical properties. The separated components are identified on the basis of the retention time determined for each of the columns. Quantitation is accomplished through area percent data obtained from a flame ionization detector (FID). The quantitative result is based on a minimum of a three-point calibration curve, which uses the peak area ratio between the analyte and the internal standard.

4.1.3 EQUIPMENT

4.1.3.1 Perkin Elmer Auto System XL Gas Chromatograph (GC)

4.1.3.2 Columns

4.1.3.2.1 Restek Rtx®-BAC1 (#18003: 30 meter X 0.32mm inner diameter (ID), 1.8µm film thickness (FT)) or equivalent column

4.1.3.2.2 Restek Rtx®-BAC2 (#18002: 30 meter X 0.32mm ID, 1.2 µm film thickness (FT)) or equivalent column

4.1.3.3 Perkin Elmer HS-40 or HS-110 Headspace Autosampler (figures 2 and 3)

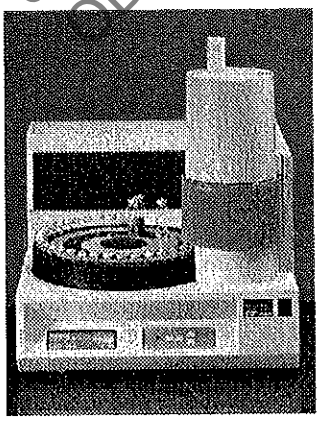


Figure 2. HS-40

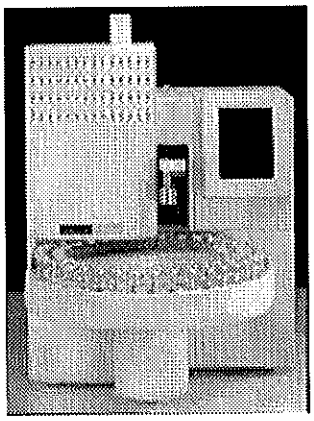


Figure 2. HS-110

4.1.3.4 PE Workstation Software, TotalChrom Version 6.2.0 or more recent version/upgrade.

4.1.3.5 Hand Crimper (P-E B003-8134 or equivalent)

4.1.3.6 Hamilton MICROLAB 503A or equivalent semi-automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250µL and 2000µL, respectively.

4.1.3.7 Glassware

4.1.3.7.1 GC-Headspace vials (P-E B010-4236 or equivalent)

4.1.3.7.2 Safety Closures {PTBE septa, crimp caps and star springs} (P-E BO10-4240 or equivalent)

4.1.4 CONTROLS AND CALIBRATORS

4.1.4.1 Whole Blood Ethanol Control (LiquiSP$_x$™ or equivalent).

4.1.4.2 Aqueous Ethanol Standards (g/100mL)
0.025, 0.05, 0.08, 0.10, 0.20, 0.30, and 0.40 (Cerilliant or equivalent)

4.1.4.3 Multicomponent alcohol Calibration Kit (Cerilliant #A-054 or equivalent)

4.1.5 REAGENTS

4.1.5.1 1-Propanol (Acros/Fisher Scientific # 23207-0010, #A996-1 or equivalent)

4.1.5.2 Acetone (Fisher #A929-1 or equivalent)

4.1.5.3 Acetaldehyde (Fisher #01004-250 or equivalent)

4.1.5.4 Isopropanol (2-Propanol) (Fisher #A416-500 or equivalent)

4.1.5.5 Methanol (Fisher #A454-1 or equivalent)

4.1.5.6 Ammonium Sulfate (Fisher #A702-500 or equivalent)

4.1.5.7 Sodium Fluoride (Fisher #S299-500 or equivalent)

4.1.6 SAFETY CONCERNS

4.1.6.1 Blood samples should be processed according to safety guidelines in the *Chemical Hygiene and Safety Manual.*

4.1.7 REAGENT PREPARATION

Record the preparation of all reagents on reagent log.

4.1.7.1 Internal Standard Solution
{0.03g/dL 1-propanol in 1.0M $(NH_4)_2SO_4$}

4.1.7.1.1 1.0M $(NH_4)_2SO_4$
Dissolve 132.14g $(NH_4)_2SO_4$ in distilled water. Dilute to 1L.

4.1.7.1.2 0.03g/dL 1-propanol in 1.0M $(NH_4)_2SO_4$

- Add approximately 800mL of 1.0M $(NH_4)_2SO_4$ to a 1000mL volumetric flask.
- Add 1g sodium fluoride *{optional}*.

- Add 375µL 1-propanol. QS to 1000mL with 1.0M $(NH_4)_2SO_4$.

4.1.7.1.3 *Solution is stable for 3 months.*

4.1.7.2 Volatile Standard Mix Solution

4.1.7.2.1 Add approximately 200 mL of DI water to a 250-mL volumetric flask.

4.1.7.2.2 Add the following volatiles, as indicated:

- 100 µL acetaldehyde
- 100 µL acetone
- 500 µL methanol
- 500 µL isopropanol
- 500 µL ethanol

4.1.7.2.3 QS to 250-mL.

4.1.7.2.4 *Solution is stable for 1 year.*

4.1.8 ANALYSIS PROCEDURE

4.1.8.1 General

4.1.8.1.1 Bring calibrators, controls, internal standard and samples to room temperature.

4.1.8.1.2 Gather necessary vials, closures and ancillary supplies in or near laminar flow hood.

4.1.8.1.3 Sample preparation should take place in a laminar flow hood.

4.1.8.2 Quality Control

4.1.8.2.1 Ethanol calibration standards must be run prior to the analysis of each batch of samples. A minimum of three points of calibration should be established.

4.1.8.2.2 An internal standard blank should follow the last ethanol calibrator.

4.1.8.2.3 A blood or aqueous control sample must be run after every 10 case samples. A minimum of two blood controls must be run per batch of samples.

4.1.8.2.4 Refer to package insert for manufacturer blood control ranges.

4.1.8.2.5 Values obtained from aqueous control and whole blood control samples must agree ± 10% of their target values.

4.1.8.2.6 Periodically run either the Volatile Standard Mix Solution or the Multicomponent Alcohol Calibration Kit solution to determine and monitor the retention of other volatiles of interest.

4.1.8.2.7 Record values for blood control samples in B*atch Analysis QC log.*

4.1.8.2.8 On a monthly basis calculate the mean, standard deviation, relative standard deviation (CV%) and percent accuracy of the control samples. The data will be used to generate a mean quality control chart.

4.1.8.2.9 New blood control lots should be analyzed a minimum of nine times prior to official use. Calculate the mean, standard deviation, relative standard deviation (CV%) and percent accuracy of the control samples.

4.1.8.3 Pipetter/Dilutor Set-up

4.1.8.3.1 Switch on power.

4.1.8.3.2 Display will inquire as to the sizes of installed syringes. Select the correct size for sample syringe [right] and reagent syringe [left].

4.1.8.3.3 Scroll down to volume option. Select 250µL for sample syringe [right] and 2000µL for reagent syringe [left].

4.1.8.3.4 Scroll down to speed option. Verify that syringe speed is on desired setting.

4.1.8.3.5 Prime the fluid path. Continue priming until no bubbles are observed.

4.1.8.4 Preparation of Blanks, Blood Control and Mixed Standard

4.1.8.4.1 Water Blank

4.1.8.4.1.1 Label test vial with *water blank.*

4.1.8.4.1.2 Add 2000µL DI water to labeled test tube.

4.1.8.4.1.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.2 Internal Standard Blank

4.1.8.4.2.1 Label test vial with *ISTD blank.*

4.1.8.4.2.2 Use Pipetter/Dilutor to dispense 2000µL of internal standard (ISTD) into labeled headspace vial.

4.1.8.4.2.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.3 Blood Control

4.1.8.4.3.1 Label two headspace vials for *blood control 1 and 2.*

internal standard (ISTD) into each labeled headspace vial.

4.1.8.5.3 Seal **immediately** with crimp cap.

4.1.8.5.4 Establish ethanol calibration plot with a minimum of three calibration points.

4.1.8.6 Initial Processing of Specimens

4.1.8.6.1 Open the sample submittal kit and remove the specimen's inner compartment. After inspecting and noting the condition of seals, open inner compartment (plastic tray or biohazard bag) and place laboratory number on each blood/urine/vitreous humor specimen.

4.1.8.6.2 When two blood/fluid samples are present, the samples should be labeled "A" and "B" or equivalent. Utilize sample "A" for analysis unless it contains insufficient sample.

4.1.8.7 Preparation of Samples for Analysis

4.1.8.7.1 Label two headspace vials with the laboratory number without the prefix.

4.1.8.7.2 Place one of the sample tubes or urine specimen bottle on tube rocker for at least two minutes.

4.1.8.8 Addition of blood, urine or vitreous humor sample to headspace vials.

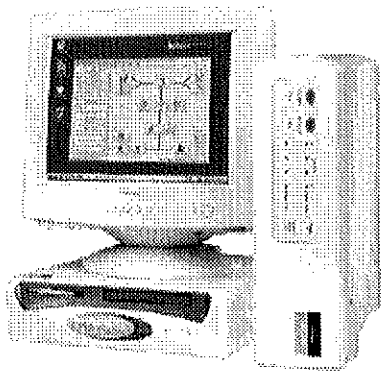
4.1.8.8.1 Use Pipetter/Dilutor dispense 250µL of sample and 2000µL of internal standard (ISTD) to a labeled headspace vial.

4.1.8.8.2 Seal headspace vials **immediately** with crimp caps as illustrated in figure 4.

4.1.8.9 Preparation for Run

4.1.8.9.1 Open **Sequence Editor**

4.1.8.9.2 Into Sequence log table, enter the sample case numbers, ethanol standards, other volatiles mix, blanks and controls.



4.1.8.4.3.2 Use Pipetter/Dilutor to dispense 250µL of blood control and 2000µL of internal standard (ISTD) into each labeled headspace vial.

4.1.8.4.3.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.4 Aqueous Controls

4.1.8.4.4.1 Label appropriate number of headspace vials for *aqueous controls (1, 2,...)*.

4.1.8.4.3.2 Use Pipetter/Dilutor to dispense 250µL of aqueous control and 2000µL of internal standard (ISTD) into each labeled headspace vial.

4.1.8.4.3.3 Seal **immediately** with crimp cap as illustrated in figure 4.

4.1.8.4.5 Mixed Other Volatiles Solution

4.1.8.4.5.1 Label test vial with *mixed volatiles.*

4.1.8.4.5.2 Use Pipetter/Dilutor to dispense 250µL of mixed volatile solution and 2000µL of internal standard (ISTD) into labeled headspace vial.

4.1.8.4.5.3 Seal **immediately** with crimp cap as illustrated in figure 4.

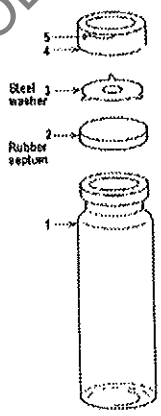


Figure 4. Crimp cap assembly

4.1.8.5 Preparation Calibration Standards

4.1.8.5.1 Label vials for standards.

4.1.8.5.2 Use Pipetter/Dilutor to dispense 250µL of appropriate ethanol concentration and 2000µL of

4.1.8.9.3 Load samples, calibration standards, blank and controls into the carousel of the headspace sampler as noted in the sequence table.

4.1.8.9.4 Active headspace sampler

- Click on the **Setup** button to open the setup instrument dialog box.
- Select sequence as the setup type, and select the desired sequence file.
- On **Setup Instrument** dialog box, designate starting and ending row.
- Verify that the paths for raw and result data files specified in the sequence indicate the desired destinations.
- Select OK in the **Setup Instrument** dialog box to initialize the instrument.

4.1.8.10 Gas Chromatography Parameters

4.1.8.10.1 Refer to instrument METHOD printout for oven program and zone temperatures. Temperature program must provide for baseline separation of volatile compounds of interest as indicated by analysis of multicomponent mixtures.

4.1.8.11 Calibration

4.1.8.11.1 Ethanol calibrators should be analyzed in order of increasing concentration.

4.1.8.11.2 The least squares line resulting from the analysis of the ethanol calibrators must have a coefficient of correlation of ≥0.995.

4.1.8.12 Acceptance Criteria

4.1.8.12.1 **Accuracy**

4.1.8.12.1.1 Qualitative

The presence of ethanol can be established if there are no significant differences in the retention time between sample and standards. The relative retention times for a specimen must be within ± 0.10 minutes of the relative retention time for the compound in question. This rejection criterion should be designated in the TotalChrom analysis method.

4.1.8.12.1.2 Quantitative
The quantitative results for a batch of samples can be accepted if the values obtained for control samples fall within 10% of their target value range.

4.1.8.12.2 **Precision**
The results obtained from duplicate analysis must agree within 0.015g/100mL. If this precision requirement is not met, the sample is reanalyzed.

4.1.8.13 Reporting of Results

4.1.8.13.1 **Blood**
Samples are quantitated to three significant figures. Report truncated mean value, of grams of ethanol per 100cc of whole blood, to two significant figures.

4.1.8.13.2 **Urine**
Samples are quantitated to three significant figures. Result obtained from blood alcohol curve should be multiplied by 0.67. Report truncated mean value, as grams of ethanol per 67 mL of urine, to two significant figures. A warning statement such as *Urine results may be of questionable value*, must be included in the report.

4.1.8.13.3 **Vitreous Humor**
Samples are quantitated to three significant figures. Report truncated mean value, as grams of ethanol per 100mL of vitreous humor, to two significant figures.

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4.1.9 QUALITY ASSURANCE

4.1.9.1 Blood or vitreous samples are to be refrigerated while at the laboratory. Urine samples can be either refrigerated or frozen.

4.1.9.2 Refer to toxicology manual section 5.1 for pipette calibration options.

4.1.9.3 Refer to toxicology manual section 5.2 for balance calibration requirements.

4.1.9.4 Refer to toxicology manual section 5.3.2 for GC-HS maintenance schedule.

4.1.9.5 Blood calibrators should be ordered prior to the current supply running out. This will allow for the analysis of new lots against existing calibrators.

4.1.10 REFERENCES

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4.1.10.9 Christmore, D.S., Kelly, R.C. and Doshier, L.A. *Improved Recovery and Stability of Ethanol in Automated Headspace Analysis,* J. Forensic Sci. 29(4): 1038-1044; 1984.

4.1.10.10 Restek Applications Note #59598, Dual-Column Confirmational GC Analysis of Blood Alcohols Using the Rtx®-BAC1 and Rtx®-BAC2 Columns Optimized for the Perkin-Elmer HS-40 Headspace Autosampler, 1999.

Idaho State Police
Forensic Services
Toxicology Section
Section Four

Blood Volatiles Determination

4.1 Quantitative Analysis for Ethanol and Qualitative Analysis for Other Volatiles by Dual Column Headspace Gas Chromatography

Revision #	Issue Date	History
0	10/01	Original Issue
1	05-15-02	Clarifications, coefficient of correlation change for system compatibility.

Approval

Technical Leader: S C Williamson **Date:** 05/15/02

Issuance

QC Manager: Rick D. Groff **Date:** June 7, 2002

Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.2 ANSYS® Thin Layer Chromatography (TLC) Methods

Standard Operating Procedures for TLC
Refer to methods listed below.

2.2.1 Toxi-Lab® Toxi-A Drug Detection System
- 2.1.1.1 Toxi-Lab® Toxi-A Instruction Manual

2.2.2 Toxi-Lab® Toxi-B Drug Detection System
- 2.2.2.1 Toxi-Lab® Toxi-B Instruction Manual

2.2.3 Toxi-Lab® Sympathomimetic Amine Differentiation
- 2.2.3.1 Toxi-Lab® Amine Differentiation with Acetaldehyde
- 2.2.3.2 Toxi-Lab® Amine Differentiation with Acetone

2.2.4 Toxi-Lab® THC II-PLUS 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (Δ9-THC-COOH) Detection System
- 2.2.4.1 Toxi-Lab® THC II-PLUS Instruction Manual
- 2.2.4.2 Summary Procedure with Controls

2.2.5 Appendix I. (Separate Binder for Available but Seldom Used Methods)
- 2.2.5.1 Toxi-Lab® Benzoylecgonine: Extraction and Detection
- 2.2.5.2 Toxi-Lab® Benzodiazepines: Hydrolysis Procedure
- 2.2.5.3 Toxi-Lab® Opiate Procedure
- 2.2.5.4 Toxi-Lab® Carbamates: Confirmation of Meprobamate and Carisoprodol with Furfural
- 2.2.5.5 Toxi-Lab® Methaqualone: Confirmation with Sodium Borohydride