

Office of Alcohol Testing
Arkansas Department of Health
Public Health Laboratory
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**BLOOD ANALYSIS
STANDARD OPERATING PROCEDURES**

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OFFICE OF ALCOHOL TESTING

BLOOD ANALYSIS

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I. General Purpose Statement

The Public Health Laboratory serves the state of Arkansas by providing analytical services, training and education, and leadership in public health practice.

Office of Alcohol Testing Profile and History

The Office of Alcohol Testing, originally named the Division of Blood Alcohol, was established by Act 106 of 1969 as amended. Other pertinent legislation includes Act 246 of 1957 as amended (ACA 5-65-201 through 5-65-207) and Act 518 of 1995 as amended.

Program guidelines were based upon recommendations of the federal government to establish an independent regulatory agency not directly responsible to individuals or agencies of the law enforcement community in order to apply unbiased regulations with no opportunity for influence from individuals being regulated. In order to accomplish this, Arkansas, like most of the states, placed this agency in the State Department of Health.

These laws specifically provide that the Arkansas Board of Health is authorized in the following areas:

- adopt appropriate rules and regulations to carry out the intent and purpose of the law;
- methods of chemical analysis for blood, urine, or breath are to be approved by the State Board of Health, Arkansas Department of Health;
- individuals (law enforcement personnel) performing such tests are to be determined to be competent and permitted (certified) to do so by the Arkansas Department of Health;
- to limit by its rules the number of types of testing devices which may be approved;
- each evidentiary breath test device is to be certified by the department every three months.

Mission

The primary role of the Office of Alcohol Testing is to serve the interest of the public in terms of individual health and the health of the community by reducing drinking and driving incidents. This mission is accomplished by:

- Providing for legally and scientifically sound tests for determining the concentration of alcohol in the human body
- Assisting the law enforcement agencies working to eliminate the problem of the drinking driver
- Guarding the public against false or unfair charges

Laboratory

Laboratory analysis of blood samples for alcohol originated at ADH in 1970 along with the regulatory program and has become a service which law enforcement and the courts system have come to respect and depend upon. Approximately ninety percent of the samples analyzed are requested by law enforcement agencies. The remaining approximate ten percent of the samples are privately requested by the subject for potential use as defense evidence as allowed by the law. The Office of Alcohol Testing laboratory is the only laboratory in the state that routinely provides this service for forensic purposes for the entire state.

Other activities of the laboratory staff include:

- Performing on-site inspections of certified instruments at law enforcement agencies
- Proficiency testing for certified instruments which includes sample preparation and QC testing
- Analyzing quality control standards subject to approval
- Downloading of breath test data from certified instruments
- Computer monitoring of instrument performance
- Preparation and QC analysis of training samples

- Reviewing current scientific literature
- Providing expert courtroom testimony relating to analysis of samples and results of inspections for both prosecution and defense
- Performing special research projects
- Evaluating new breath testing equipment
- Evaluating new alcohol testing procedures and protocols
- Breath testing equipment repair/ maintenance

Training

The Office of Alcohol Testing establishes training standards and training course content for law enforcement personnel for alcohol testing. Law enforcement officers are trained each year to operate certified breath testing instruments in order to assure accurate alcohol test results.

Certification/ Administration

Arkansas law requires that the Arkansas Department of Health provide for the certification of breath testing devices in order to assure accurate alcohol test results. Necessary components leading to a successful certification program include:

- Establishing requirements and protocol for sample collection and approval of methodology and equipment used for alcohol testing
- Designing technical standards in keeping with current scientific community standards for alcohol testing as well as legal mandates and nationwide trends
- Establishing a method of record keeping and defining records retention
- Ongoing revision of regulations

Other Legal Mandates

The Office of Alcohol Testing has other legally mandated responsibilities that include:

- Adoption and implementation of regulations for breath alcohol ignition interlock devices and inspections (Act 298 of 1993)
- Assistance to the Office of Driver Services in the hearing process on Administrative License Revocation resulting from DWI arrests (Act 802 of 1995)
- Repair service for locally owned breath test instruments (Act 577 of 1989)

II. Regulations

The *Arkansas Regulations for Alcohol Testing* outlines the requirements for the following functions of the Office of Alcohol Testing: certification, training, sample collection and handling, methods of analysis, and records and reporting.

Please refer to this document (which can be obtained by calling the Office of Alcohol Testing) to find that:

The following regulations for alcohol testing are duly adopted and promulgated by the Arkansas Department of Health as approved by the Arkansas State Board of Health pursuant to the authority expressly conferred by the laws of the State of Arkansas, Act 106 of 1969 as amended and Act 346 of 1957 as amended, the same being Arkansas Code, Title 5, Chapter 65 and Act 518 of 1995 as amended.

III. SDS/ COA Location

All SDS (Safety Data Sheet) and COA (Certificate of Analysis) paperwork are kept on file in the OAT Laboratory or the ADH Stockroom. The AR-PHL safety manual can be found in the OAT office area.

IV. Gas Chromatography (GC) Principle

Gas Chromatography (GC) is used to separate volatile components of a mixture. Bodily fluid samples are analyzed for ethanol by headspace gas-chromatography.

V. Agilent GC Instrumentation

a. Introduction

The Office of Alcohol Testing is part of the Public Health Laboratory (PHL). Please refer to the PHL Clinical Quality Assurance Plan and Safety Plan for QA and Safety documents.

The Agilent 7890B Gas Chromatograph (GC) utilizes a dual column / dual flame-ionization detectors (FID) system to measure ethanol. The sampling is performed using an Agilent 7697A Headspace Sampler. The injections are performed by automation using the GC Chemstation. Per manufacturers parameters the room temperature must be within 15-35°C. This is checked each day and recorded on the temperature log (PHL-13-30).

Note: If the temperature falls outside the acceptable range notify the supervisor. No analysis can be done until temperature is within acceptable limits.

An aliquot of each biological specimen is diluted with an internal standard solution into a glass vial, sealed, and placed in a heated headspace sampler. The concentration of ethanol in a dilute aqueous biological sample is directly proportional to the concentration of ethanol in the gas phase (headspace). A portion of the resultant headspace vapor above the liquid is automatically injected into a dual column gas chromatograph (GC) equipped with dual flame ionization detectors (FID). Ethanol and n-propanol are identified by retention time. The ethanol concentration is calculated automatically by the software based on linear regression of the calibration curve.

The GC is calibrated using a series of known solutions. The Chemstation correlates the response of the detectors with each known amount, and then automatically generates a calibration curve. The resulting calibration curve is then used to determine the amount of alcohol in analytical samples.

Various aqueous and blood solutions are tested with each day's analysis as part of the calibration checks. These solutions are verified through the quality assurance testing procedure prior to use.

b. Gases/ Gas Leakage Check

Gas Chromatograph Operational Parameters. The following conditions are recommended starting parameters. Instrument conditions may be adjusted to permit improved performance. Nitrogen is used as the carrier gas. Hydrogen and compressed air are the fuel for the FIDs.

Gas tanks should be changed when the tank pressure falls below 500 psi to prevent the introduction of impurities into the system.

The FID gases should be turned OFF at the end of a day of analysis to conserve gas.

Whenever a tank is changed out it must be labeled with the date it was put into service and a leak check at connections must be done. Using a soapy solution squirt a small amount around the connections- if the liquid bubbles up then there is a leak and the connection needs to be tightened. If there are no bubbles the connection is good. Record the tank change and leak check on the tank change form (PHL-13-31).

The connections should be checked if the tubing or connections are manipulated too. That should be recorded on the same form.

Compressed air must be at least breathing grade quality; nitrogen and hydrogen must be ultrahigh purity grade.

c. Chemstation

The GC and sampler are automated through the use of Chemstation. Chemstation contains three main applications:

- Method and Run Control
- Data Analysis
- Reports

Chemstation controls instruments, automates data collection and analysis, and documents the results on the hard drive and printed reports.

Method and run control are where live injections and calculations are performed. Chemstation provides data-handling parameters for peak integration and result calculations. The parameters are stored in the method.

Chemstation records the method, raw data, calculated results, and analytical conditions for every chromatographic run. It also makes a record of the GC functions during each run. Using this stored information, Chemstation can automatically print a variety of reports, including the calculated results and the chromatogram for each run. Customized reports are printed for each injection.

d. Calibration Procedure

Agilent allows for the calibration performed by each chemist to be saved separately (the calibration is not replaced when another chemist analyzes samples). Therefore the same calibration may be used to run samples on different days. However, a new calibration must be performed monthly by a chemist if they have samples to analyze.

The standards used in calibrating the instruments are obtained from Cerilliant. The standards used in performing a calibration are: .025, .080, .150, .200, .300, and .400 (g/dL). These standards are traceable to NIST Standards and come with a Certificate of Analysis. A blank will be used as the first level. Each of the standards is assigned a calibration level which will be used for calibration verification purposes during analysis of other solutions. All samples are prepared and analyzed in the same manner described in the Agilent GC Analysis section of this SOP.

Once the calibration is run, print out the calibration graph using linear regression. The r^2 value must be equal to or greater than .9995.

To print curves and save calibration to a specific analysts master method:

- After the calibration is completed click on the Data Analysis tab in the lower left portion of the Agilent control screen.
- To print Calibration Curves:
 - Select the calibration run just completed
 - Click on the file tab at the top, hover over print, and select "Calib Table + Curves"
- To save the calibration to the analysts Master Method:
 - Check the calibration curves just printed to confirm that calibration is acceptable.
 - Click on the Method tab and then click on "update Master Method".

Attach the calibration printouts to the analysis done that day and give to the supervisor for review.

e. Calibration Sample List

Line	Sample Name	Sample Type	Cal Lev	Inj.	Sample Information	Sample Location
1	Blank	Calibration	1	1		1
2	.025 CER	Calibration	2	1	CER Lot # and Expiration Date	2
3	.080 CER	Calibration	3	1	"	3
4	.150 CER	Calibration	4	1	"	4
5	.200 CER	Calibration	5	1	"	5
6	.300 CER	Calibration	6	1	"	6
7	.400 CER	Calibration	7	1	"	7

f. Maintenance Records

Whenever maintenance is performed on the GC it must be recorded on the Equipment Log sheet (PHL 12-113). This sheet can be found in the filing cabinet in the GC lab. The supervisor will review. The manufacturer will visit annually for a PM visit. This will be logged and then passed to supervisor for review. Anytime maintenance is performed the supervisor must review the records.

g. GC Column Verification

Whenever a column is changed a new calibration must be performed. Five samples previously analyzed must be analyzed after this calibration and the results must agree within +/- 0.010 of the originally reported results. Pass to supervisor for review. The column change and results of sample analysis must be noted on the equipment log sheet (PHL 12-113).

h. GC Calculation Verification

The calculation performed by the GC must be manually verified every two years. If the results do not match those on the computer notify the supervisor. Documentation for calculations is found in the cabinet in the GC lab.

i. Carryover Detection & Analytical Interferences

Potential carryover and analytical interferences are addressed through the analysis of a MIX sample. This sample does not contain ethanol. A sample is ran as part of the QC standards and a sample will be ran as the last sample of each run. MIX is a mixture sample of acetone, iso-propanol, and methanol. There should be no ethanol result for this sample. If a result for ethanol is found the analysis will be stopped and the supervisor notified.

j. Column and Detector Monitoring

The column and detector are monitored each run by the analysis of quality control standards. If the expected result is not obtained the supervisor will review the data for operator error, column retention time information, and elution of peaks to determine what course of action should be taken. Any needed maintenance must be documented on the equipment log PHL 12-113.

k. Reportable Range and Analytical Sensitivity

The reportable range is 0.000-0.400. Per the approved method validation, the lower limit of the analytical sensitivity is not applicable, since the results obtained from analysis of samples are introduced into court for legal proceedings, a result must be given. OAT cannot report a less than value.

I. Instrument Troubleshooting

Minor troubleshooting and repair of the instrument guidelines can be found in manuals or by calling the manufacturer.

m. Reagent Expiration Dates

Unless noted by the manufacturer, liquid reagents expire 5 years from opening. All reagent bottles must be labeled with the received, opened, and expiration date. Once the expiration date has been reached the new expiration date will be five years later provided that all solutions prepared with the reagents test within acceptable limits.

n. AMR Verification

Every six months the analytical measurement range (AMR) must be verified using an approved 0.048, 0.181, and 0.362 solutions. The 0.048 and 0.181 are samples routinely prepared and approved for use in the lab as standards when analyzing legal samples by GC. The 0.362 solution can be obtained by double pipetting the 0.181 solution. The results of these analyzes must be within +/- 0.010 of the expected value. The chemist performing the verification will analyze these samples in conjunction with legal samples during a routine run.

If the results are not within acceptable limits a new calibration curve must be ran and the appropriate samples re-analyzed. A corrective action must be initiated.

Once completed pass all paperwork the supervisor for approval. Results will be filed in the GC lab cabinet.

VI. Agilent GC Procedure

a. GC Solution Preparation

Note: **Solutions are to be prepared in the GC Lab only**

PPE Required: gloves, goggles, and a lab coat.

For the following solutions use only distilled water

All solutions used must be at least Reagent Grade

Unless noted, solutions are made as needed and expire three years from preparation date.

Label each with: initials, solution content, concentration, preparation date, and expiration date

- Each bottle must be labeled with the proper storage temperature 2-8 C

Store bottles in cold room.

New solutions will be analyzed by GC using the current approved standards for comparison testing
Preparation, analysis, and supervisor approval to be documented at:

\\phlfs\PHL_Share\OAT\Documents\NewSolutionApproval.

Internal Standard (0.03% n-prop)

1. Partially fill a 2 liter volumetric flask with distilled water. Pipette 0.6mL of n-propanol into the flask and dilute to the mark with distilled water.
2. Mix well and pour the solution into plastic bottles so that there is no head space left.
3. Discard any previous n- prop solution
4. The new internal standard is verified by doing a new calibration and running standards using the new solution.

NOTE: Solution expires two years from preparation.

Mix Solution

1. Partially fill a 1000ml volumetric flask with distilled water, add 0.4ml of each: acetone, iso-propanol, and methanol, and dilute to the mark with water.
2. Mix well and pour the solution into plastic bottles so that there is no head space left.
3. When preparing note the lot numbers of the acetone, iso-propanol, and methanol on preparation paperwork. The new solution will be named Mix-MM/YY.
 - Example: Mix prepared in March 2014 will be Mix 03/14
4. MIX is verified by GC analysis.

Blank (to be used in GC calibration procedure)

1. Pipette 150 uL of internal standard to a clean, dry GC vial
2. Pipette 50 uL of distilled water into the same vial
3. Cap and vortex

.048CS

1. Partially fill a 1000ml volumetric flask with water, add 1 ampoule of .08% certified stock solution, and dilute to the mark with water.
2. Mix well and pour the solution into plastic bottles so that there is no head space left.
3. .048CS is verified by GC analysis.

.121CS

1. Partially fill a 1000ml volumetric flask with water, add 2 ampoules of .10% certified stock solution, and dilute to the mark with water.
2. Mix well and pour the solution into plastic bottles so that there is no head space left.
3. .121CS is verified by GC analysis.

.181CS

1. Partially fill a 1000ml volumetric flask with water, add two ampoules of .15% certified stock solution., and dilute to the mark with water.
2. Mix well and pour the solution into plastic bottles so that there is no head space left.
3. .181CS is verified by GC analysis.

b. Analysis Procedure

1. Check compressed gas tank pressures. Change any tanks that are at or below 500 psi.
2. To activate the Agilent GC system:
 - Click on the GC Open Lab icon.
 - Log-in
 - Under Choose Method Load Option select Download to Instrument
 - Method and gases will be activated
 - Verify that the date and time on the PC and GC are correct.
3. Chose the appropriate analyst method and open the appropriate sequence table (sample list). Edit the sequence for the proper calibration, verification, proficiency, certified stock solutions, and/ or legal blood/ urine samples to be analyzed.
 - Examples of each type of run and the correct standards used (and an example of each sample list) follow at the end of these instructions.
 - Different types of samples may be analyzed at the same time as long as the correct standards are analyzed at the beginning of the run.

NOTE: If the automated sequence is left to run unattended, any .121CS sample result not within limits invalidates the next ten sample vials. A second injection may be performed on the sample vials (on the same day) after the verification standard is repeated with good results. If the repeat cannot be performed on the same day then the complete analysis will need to be repeated.

4. Print sample list as a reference for labeling vials.

NOTE: the initial sample preparation procedure is the same for any sample that is analyzed.

5. Label the vials with standard ID and/or legal sample numbers.

SAFETY NOTE: All blood/ urine samples are to be pipetted from behind the biohazard shield, using sleeves and gloves for personal protection equipment (PPE).

6. Make sure to pipette directly to the bottom of the vial. When pipetting samples, make sure to minimize open air time of the solutions and samples. When vortexing a sample, make sure to do so gently that the sample stays at the bottom of the vial. This will minimize contamination of the inlet and column.
7. Pipette 450 uL of the 0.03% n-propanol internal standard solution into a vial and place the cap on top.
8. Pipette 50 uL of each of the standards and legal samples, using a new pipette tip each time, into the appropriate target vial, cap, seal with the crimper, and vortex gently.

NOTE: If there are two or more tubes of the same sample, use one tube for one duplicate and a second tube for the second duplicate. Pipette one sample at a time to prevent cross contamination of specimens.

9. Gently place the samples in the proper vial position in the sample tray of the headspace sampler.

IMPORTANT: Make sure that the vials in the sample tray are in the position number indicated on the sample list.

10. To start analysis: hit run.
11. If any sample results are above the calibration limit (0.40 % w/v) that sample shall be diluted by half and reanalyzed as follows:
 - Obtain and label a mixing tube with stopper for each blood tube submitted.
 - Pipette 200 uL of distilled water into each tube(s).
 - Pipette 200 uL of the sample into each tube(s).
 - Thoroughly vortex each tube(s).
 - Repeat steps 8 through 10 using each mixing tube in place of the original sample.
 - Multiply results by two for reporting purposes when diluting the sample by half.

NOTE: A sample may only be diluted one time to obtain a result.

12. Once analysis is completed, turn off the hydrogen and air on the front panel of the Agilent GC.

c. System Inoperable

If at any time the GC becomes inoperable notify the supervisor. If repairs cannot be done in house then Agilent will be contacted to set up a repair visit. Notes of problems/ repairs will be written up and filed in the GC lab. Document this information on the equipment log sheet. Notify the QA Director and Lab Director of any delay (more than one month) in reporting.

d. Sample Storage

All samples (ethanol positive and ethanol negative) are stored in the walk-in cooler in the GC lab. The temperature range is 2-8°C. If the temperature falls outside that range then an alarm will sound (the cooler is electronically monitored). Contact supervisor if this happens.

The temperature is to be recorded daily on the Temperature Log (PHL-13-30). On weekends or when the office is closed- record TWR (temperature within range) as long as maintenance does not report that a temperature violation occurred.

e. Certified Stock Solutions

Calibration and Verification Standards to be analyzed are:

- .04 CER Standard (in triplicate)
- .10 CER Standard (in triplicate)
- .07BS (run current std when approving new std)
- MIX
- .121CS
- Samples Submitted for Approval (in triplicate)
 - Blood standards
 - Pre-mix/ Ampoules
 - Certified calibration checks

MIX is a mixture sample of acetone, iso-propanol, and methanol. There should be no ethanol result for this sample.

Acceptable results must fall within the following limits of the target values:

- a) Averages of samples submitted for approval (except for blood std): $\pm .005$
- b) .07 BS and .121CS: $\pm .010$
- c) Average of CER Standards: $\pm .005$
- d) Average of certified Calibration Solutions: $\pm .005$

The Agilent GC utilizes a dual column system- resulting in two results per sample analyzed. For reporting purposes the lowest result of each sample will be recorded as the final result.

If at any time the results of the calibration/ verification standards fall outside of the limits of the target values then the analysis must be stopped. Replace the standard that fell out of range and begin the automation sequence at that selected point. If the results of the last standard of the run fall outside the limits the samples ran before that analysis must be repeated.

NOTE: Regardless of lot number, each shipment of the .07 BS must be approved for use.

If any results fall outside of the acceptable limits, then the sample must be re-analyzed. If analysis fails a second time notify supervisor.

i. Certified Stock Solution Sample List

Line	Sample Name	Sample Type	Cal Lev	Inj.	Sample Information	Sample Location
1	.04 CER	Control Sample		1		1
2	.04 CER	Control Sample		1		2
3	.04 CER	Control Sample		1		3
4	.10 CER	Control Sample		1		4
5	.10 CER	Control Sample		1		5
6	.10 CER	Control Sample		1		6
7	Mix	Control Sample		1		7
8	.121CS	Control Sample		1		8
9	Sample A	Sample		1		9
10	Sample A	Sample		1		10
11	Sample A	Sample		1		11
12	Sample B	Sample		1		12
13	Sample B	Sample		1		13
14	Sample B	Sample		1		14
15	Sample C	Sample		1		15
16	Sample C	Sample		1		16
17	Sample C	Sample		1		17
18	Sample D	Sample		1		18
19	.121CS	Control Sample		1		19
20	Sample D	Sample		1		20
21	Sample D	Sample		1		21
22	.121CS	Control Sample		1		22
23	Mix	Control Sample		1		23

f. Legal and Proficiency Samples

Calibration Verification Check Standards to be analyzed are:

- .04 CER
- .10 CER
- .048CS
- .07BS
- .181CS
- MIX
- .121CS
- Samples Submitted for Analysis

All legal samples are to be analyzed in duplicate; the lowest result is truncated and reported.

Enter the subjects name under the injection notes column.

Acceptable results must fall within the following limits of the target values:

- a) Calibration verification check standards: ± 0.010
- b) Legal sample duplicates are to be within ± 0.010 of each other.
- c) External proficiency duplicates are to be within ± 0.010 of each other.
- d) Results of CER standards: ± 0.005 of target

The Agilent GC utilizes a dual column system- resulting in two results per sample analyzed. For reporting purposes the lowest result of each sample will be recorded as the final result. For legal samples analyzed in duplicate the lowest result of each column will be compared for acceptability.

If at any time the results of the calibration verification checks fall outside of the limits of the target values then the analysis must be stopped. Replace the sample that fell out of range and begin the automation sequence at that selected point. If the results of the last standard of the run fall outside the limits the samples ran before that analysis must be repeated.

When analyzing a urine sample, final results are obtained by dividing the original result by 1.3, in accordance with "Arkansas Regulations for Blood Alcohol Testing", 4.41. This must be written on the chromatogram. That result will be used in all documentation.

NOTE: This does not apply to the in-house urine proficiency process- this process reports the numbers reported by the GC.

Proficiency samples are to be analyzed in the same manner as legal samples and should be included in a run with legal samples when possible.

If any results fall outside of the acceptable limits, then the sample must be re-analyzed.

NOTE: If a legal sample is clotty and analyzed three times without results being within acceptable limits, then the lowest of the six analyses may be reported with supervisor approval.

i. Legal and Proficiency Sample List

Line	Sample Name	Sample Type	Cal Lev	Inj.	Sample Information: Last Name, First, Middle	Sample Location
1	.04CER	Control Sample		1		1
2	.10CER	Control Sample		1		2
3	.048CS	Control Sample		1		3
4	.07BS	Control Sample		1		4
5	.181CS	Control Sample		1		5
6	MIX	Control Sample		1		6
7	.121CS	Control Sample		1		7
8	Sample A	Sample		1		8
9	Sample A	Sample		1		9
10	Sample B	Sample		1		10
11	Sample B	Sample		1		11
12	Sample C	Sample		1		12
13	Sample C	Sample		1		13
14	Sample D	Sample		1		14
15	Sample D	Sample		1		15
16	Sample E	Sample		1		16
17	Sample E	Sample		1		17
18	.121CS	Control Sample		1		18
19	Sample F	Sample		1		19
20	Sample F	Sample		1		20
21	Sample G	Sample		1		21
22	Sample G	Sample		1		22
23	Sample H	Sample		1		23
24	Sample H	Sample		1		24
25	Sample I	Sample		1		25
26	Sample I	Sample		1		26
27	Sample J	Sample		1		27
28	Sample J	Sample		1		28
29	.121CS	Control Sample		1		29
30	MIX	Control Sample		1		30

g. Logging in Results

The results of all calibration verification check samples are to be recorded in the computer in excel at:
\\phlfs\PHL_share\OAT\Documents\ GC-QC Worksheets.

Open up the corresponding folder and there is a file for each standard. Enter the data into the appropriate file.

The computer will automatically calculate the mean and the standard deviation for each page:

For the systematic error (SE), the coefficient of variation (CV), the average mean of the last four pages, and the average standard deviation of the last four pages to be calculated, you will need to enter the appropriate numbers from the previous page at the bottom of the current one. Once this is done, the computer will calculate the control limits as defined by $\pm 1s$, $\pm 2s$, and $\pm 3s$. An * next to your result will indicate the result is outside that control limit.

Observe the results and watch for trends that could signal significant changes. Values that signal the process is Out Of Control include:

- a) one or more points outside of 3s
- b) two or more consecutive points outside 2s
- c) a run of four points outside 1s
- d) a run of seven or more points on one side of the mean or a run of 7 or more points increasing or decreasing

If the above trends are observed, but the values are within the +0.010 w/v accuracy requirement, report this trend to the supervisor. Note the trend under the remarks column for that line.

The results of all proficiency and certified stock solutions are to be recorded at either:
\\phlfs\PHL_share\OAT\Documents\ Proficiency Reports\Proficiency Prep Analysis or \ New Solution Approval.

The results of legal samples are to be recorded in Agilent results page at
\\phlfs\PHL_share\OAT\Documents\ GC-QC Worksheets. The computer program will automatically calculate the mean deviation and average mean deviation for the last four pages in the same manner as described above for the standards.

VII. Proficiency Samples: Internal and External

OAT participates in blood alcohol proficiency testing through the College of American Pathologists (CAP):

- When the samples are received by lab personnel, the QA office must be notified so that the sample can be entered into the QA proficiency tracking system.
- CAP samples have a unique identifier so an OAT sample number is not assigned.
- The preparation and analysis of the blood proficiency samples are the same as found in the GC Analysis section of this SOP.
- When the analysis has been completed, the results will be submitted to the supervisor, QA office, and lab director for approval. Once approved the results can be submitted.
- CAP will report a Satisfactory/ Unsatisfactory when returning results of the proficiency test.
- When the results are returned, the supervisor will notify the lab and QA office as to the results of the proficiency samples. Copies of the vendor report will be passed to the QA office for documentation.
- The proficiency tracking system will then be updated. In the event of an Unsatisfactory the supervisor will start a corrective action.

No commercial proficiency test is available for quantifying ethanol in urine. OAT will utilize this method:

- Prior to the proficiency tests, multiple internal urine samples are prepared in the laboratory and analyzed ten times to determine the mean target result.
- At the time of the proficiency tests the supervisor will aliquot two of the already prepared and analyzed samples.
- The QA office must be notified so that the sample can be entered into the QA proficiency tracking system.
- The samples will be given a unique identifier so an OAT sample number is not assigned.
- The analysis of the urine proficiency samples are the same as found in the GC Analysis section of this SOP.
- The analyst performing the analysis will be blinded to the target result of the sample.
- When the analysis has been completed, the results will be submitted to the supervisor and QA office for approval. Once approved the results can be submitted.
- To pass the PT the analysis must be within +/- .010 of the target. If the result is outside that then it is considered Unsatisfactory.
- The proficiency tracking system will then be updated. In the event of an Unsatisfactory the supervisor will start a corrective action.
- This proficiency testing must be done twice per calendar year.

VIII. Corrective Actions

Corrective actions will be written for any of the following errors:

- Improper storage of samples
- Failure to comply with SOP requirements
- Proficiency test failure
- Reporting of incorrect results
- Results of QC samples exceed tolerance

Corrective actions are initiated and tracked at the following site:

<http://adhwebprod12/labcorrectiveaction/CAstart>.

IX. Sample Documentation

a. Sample Log In

NOTE: by law all samples must be analyzed (regardless of collection container or lack of labeling) as long as the sample meets certain criteria. Because of the nature of these samples there is no option for re-collection.

PPE required: eye protection, lab coat, and gloves

Log all samples received in the Blood Sample Log. Be sure to note the following:

- Date received
- Note the type of container in which the sample was received.
 - EXAMPLE: C = carton, T = tube, B = box.
- Via (how it was received):
 - If the sample is hand delivered, note, "del".
 - If it arrives in the Blood Alcohol mailbag, note "8509"
 - If it arrives through the mail by any other route, note "mail."
 - If it arrives by certified or registered mail, abbreviate "c.m." or "reg.m." Log the certified or registered mail by recording the last four digits of the number in the remarks colu.mn.
- Subject's name, last name first.
- Assign the next consecutive sample number. Clearly note the complete sample number on each sample tube(s), in the appropriate space on the Blood Alcohol Report Form (BARF), and on all other documents received. If more than one tube is received make sure to note this in the Sample Analysis and Result section of the BARF.
- Check (✓) if the sample is a private sample.
- The Remarks/ Release/ Transfer Information on the Blood Sample Log are used in recording any useful information about the sample. If the sample is mailed back to the officer, or transferred elsewhere for analysis, the information is recorded here, along with the date and initials of the person transferring the sample.
- Security of Samples: The samples are to be secured/ locked in the walk in cooler prior to preparation for analysis. After analysis, return the tubes to the cooler and store in the upright position. Samples are retained for a minimum of two years. When destroying old samples, document it in the Blood Sample Log.

b. Completion of Blood Alcohol Report Form (BARF)

NOTE: If a sample is received without a BARF the sample will not be given a sample number and will be returned to the submitter. A copy of the sample return documentation will be placed in the binder in the GC lab.

The chemist logging the sample is responsible for checking the completion of the form up to this point.

NOTE: If any information is recorded or corrected on the form by the chemist, then that information must be dated and initialed.

If the sample meets any of the following criteria it will be rejected and returned to the submitter with a copy of the BARF and the Sample Return Letter. A sample number will be assigned.

- the sample is not traffic related
- the sample does not contain a sufficient amount of preservative
- there is not enough sample for analysis
- the sample exhibits extreme clotting
- the sample tube is broken
- the sample collection time on a post mortem sample exceeds 24 hours after death
- an alcohol and drug screen or drug screen only was requested

- the blood tube was expired at the time of collection.

If the sample submitted does not have the proper identification attach a copy of the Tube Labeling Reminder Letter to the blood form. Examples include:

- blood tube not labeled
- name on form and blood tube do not match
- unable to read label on blood tube (due to too much security tape)

Part I – Subject Information

The submitting officer is responsible for this information. All this office requires is the subject's name.

Part II- Incident Information

- The submitting officer is responsible for completing this section.
- If any information is missing, then it is to be left blank with the office not making any changes, with the following exception: if there is a question as to whether or not the sample is a law enforcement or subject request, then the chemist will ascertain the correct information and note the change.
- If the subject is deceased, a post mortem form must also accompany the sample. If the sample was drawn more than 24 hrs after death, return the sample along with the Sample Return Letter.

Part III - Collection of Blood or Urine Only Information

- The office will not make any changes to this section. The submitting officer should complete all sections.
- If sufficient sodium fluoride is not present in the blood sample (0.2-0.3% for live subjects, 1.0% for post mortem), note this information in the remarks section. The sample will not be analyzed and will be returned to the officer as outlined in part b.

Part IV - Sample Transfer Information

Note the source from which the sample was received, who received it, and the date.

- If the sample is received by certified or registered mail, record the complete number and sign the form received by Office of Alcohol Testing.
- If the sample is delivered to the lab, have the delivery person sign the form; then sign the form received by Office of Alcohol Testing.
- If the sample is received by the post office (in a sealed bag) then the sample is received from 8509 Mail to the Office of Alcohol Testing.
- If the sample is received through the mailroom downstairs, then the sample is received from ADH Mailroom to the Office of Alcohol Testing.
- If the sample is received through any other delivery service (UPS, Fed-Ex, etc.), then the sample is received from them to the Office of Alcohol Testing.

Part V - Sample Analysis & Results Information

Fill out the information describing the sample:

- The amount and type of sample must be noted along with what the sample was received in (tube, plastic vial, etc) and how the sample was labeled. Note the expiration date of the blood tube.
- If blood and any other type of fluid are received from the same subject, then only the blood will be analyzed.
- If more than one tube is received, but only one tube has the correct amount of preservative, then that tube will be analyzed. In either case, make sure to note this in this section.
- If the form used is an old revision, then this information will need to be noted in the blank paragraph.
- Record the corresponding sample number from the Blood Sample Log.

The remainder of this section is to be completed upon analysis of the sample:

- The name to be used for installation is Arkansas Department of Health (ADH may be used instead).
- The department's installation number is 001 (this is needed only on the older version of the form).
- The date and time of analysis (from the GC report printout).

- After recording the test results in the box, write the results in longhand directly below the numerical value.
- Once all the information is completed, the chemist will:
 - sign the form/ print name underneath.
 - record the result in the Blood Sample Log.
 - enter the information into the lab database.
- For newer forms with the attestation statement on the front, the following procedure for processing reports is followed:
 - The chemist analyzes the sample, assures form and all logs are completed properly, and submits the Blood Alcohol Report Form (report) with attached chromatogram(s) to another chemist for review.
 - After review route the reports to the section director to sign and stamp all form copies with the laboratory developed test (LDT) stamp. An assistant director may be designated to sign forms when needed.
- Once approved, the report is given to the secretary who separates the original from the report, staples the chromatogram(s) and any other documentation to the other copies, and files all away.
- Reports:
 - The secretary will mail the original report to the address provided on the form.
 - If it is a subject requested test then the report can only be mailed to the subject or the hospital where the sample was collected.
 - If a court order/ subpoena is received then this report can be released to a law enforcement agency or court official
 - Copies of non-subject requested samples are not subject to HIPPA and can be released to any requesting party. A fax or emailed request is required so that the request can be attached to the laboratory copy.
 - If a copy of a subject requested sample is requested by the subject then he must fax/ email a copy of this photo ID. Once that is received then the copy can be mailed or faxed and the request attached to the laboratory copy. This is per the ADH legal department.

If the report is an older revision, the chemist must sign and attach the Attestation Form and then process as above.

Sample records are located in the administration area of OAT. This is a secure area that is a limited access secured area accessible by badge. The only records subject to HIPPA are those that are subject requested. Those records are stored in a locked filing cabinet in the admin area. The key to this cabinet is maintained by the administrative personnel.

Per the Arkansas General Records Retention schedule (section GS01008) records are kept a minimum of four years. The schedule can be located at:

http://www.dfa.arkansas.gov/offices/intergovernmentalServices/Documents/rec_retention_schedule.pdf.

However, per the CAP's more stringent requirements, OAT maintains records for 10 years.

X. Analyst Training

All new hire and/or transferred analyst are placed on a six (6) month probationary period mandated by the Department of Health. During this probationary period, which may be extended if required, the training analyst will be instructed in various requirements and duties for their position. At NO time will the probationary analyst perform analysis on legal samples until they have completed the certification procedure and been approved by the section director and lab director.

The training will include the following:

- Complete Review of Standard Operating Procedures
- Sample Receiving and Log-in
- Analysts Certification Procedure for Legal Specimens
- Competency Assessment (PHL-12-100)
- AR-PHL Training Documentation (PHL 12-26)

These training criteria will be done concurrently and require significant observations and interactions with the other analysts.

The section director will complete the Analyst Training Certification Checklist to be kept on file as part of the Analyst Certification Procedure.

a. Sample Receiving and Log-in

The training analyst will observe and assist with the receiving and logging in of samples by other analysts. The training analyst will also observe the completion of the analysis report and the processing of the documents.

b. Certification Procedure for Legal Specimens

To be certified by the Agency to analyze legal samples, fifty (50) bodily fluid samples must be successfully analyzed, documented and recorded.

The training analyst will be given fifty (50) previously analyzed blood, urine or vitreous fluid samples. No more than five (5) samples that have been determined to have zero (0) alcohol content may be selected. Verify the results with the previous analysis results reported in the database for all selected samples. If necessary, additional samples may be selected in order to obtain the minimum number of acceptable sample results.

To be acceptable, the results must meet all the following criteria:

- The duplicate sample results must be within ± 0.010 of each other.
- The sample results must be within ± 0.010 of previous analysis recorded.

NOTE: To avoid confusion with legal sample runs, label all calibration verification samples with the letter “T” for training in the sample name. (See example form.)

Blank copies of the Blood Alcohol Report Form will be completed and submitted as part of the documentation. All QC/QA standard results and sample results will be logged in the Training GC worksheets as instructed in the SOP.

All documentation will be presented to the section director and lab director for approval. Upon approval the training analyst will be certified to analyze legal bodily fluid samples.

c. Certification Lists and Results Page

The following are examples of sample lists and data results forms that may be used to report the results.

i. Legal Samples

Line	Sample Name	Sample Type	Cal Lev	Inj.	Injection Notes	Vial
1	.04CER	Analysis		1		1
2	.10CER	Analysis		1		2
3	T.04CS	Analysis		1		3
4	T.08BS	Analysis		1		4
5	T.18CS	Analysis		1		5
6	TMIX	Analysis		1		6
7	T.121CS	Analysis		1		7
8	FML-1	Analysis		1	Subject Name/ Sample #	8
9	FML-2	Analysis		1	"	9
10	FML-3	Analysis		1	"	10
11	FML-4	Analysis		1	"	11
12	FML-5	Analysis		1	"	12
13	FML-6	Analysis		1	"	13
14	FML-7	Analysis		1	"	14
15	FML-8	Analysis		1	"	15
16	FML-9	Analysis		1	"	16
17	FML-10	Analysis		1	"	17
18	T.121CS	Analysis		1	"	18
19	FML-11	Analysis		1	"	19
20	FML-12	Analysis		1	"	20
21	FML-13	Analysis		1	"	21
22	FML-14	Analysis		1	"	22
23	FML-15	Analysis		1	"	23
24	FML-16	Analysis		1	"	24
25	FML-17	Analysis		1	"	25
26	FML-18	Analysis		1	"	26
27	FML-19	Analysis		1	"	27
28	FML-20	Analysis		1	"	28
29	T.121CS	Analysis		1	"	29

XI. Competency Assessment

All chemists will have a competency assessment done on a yearly basis. The competency worksheet will include pre-analytic aspects, analytic aspects, and post-analytic aspects. Results will be recorded on the competency assessment template, PHL-12-100.

For new chemists a competency assessment will be done at the 6 month mark, one year mark, and then annually thereafter.

XII. Health and Safety Warnings

- Standard laboratory protective clothing, gloves, and eye covering are required.
- Eating, drinking, applying make-up, and handling contact lens are prohibited when working with blood samples or with contaminated equipment.
- Pipetting solutions and samples by mouth is prohibited.
- All areas must be wiped down with appropriate disinfect wipes when done pipetting blood samples. Pipettes must be cleaned in the same manor. Disinfection of workspace and pipettes is documented on PHL-13-36.
- OAT lab will follow the AR-PHL Safety Manual. Copies are located in OAT Office Area and in iPassport.
- Analyst must attend general lab safety and radiation safety training annually.

XIII. Package Inserts

No package inserts are used in the laboratory.

XIV. Testing Time Frame

There is no defined time from receipt of sample to reporting of results.

XV. Thermometer Verification

The lab has two thermometers: one used for room temperature (15-35C) and one used for cooler temperature (2-8C). Each thermometer was verified against a NIST traceable thermometer prior to use, whenever performance is suspect, and will be verified annually. The procedure for verification can be found in the Clinical Laboratory Quality Assurance Plan. The data will be recorded on PHL-12-117. Each thermometer will be tagged with serial number, correction factor, date of verification, and initials of person performing verification.

XVI. Temperature Checks

The temperature checks must be done and recorded (PHL-13-30) daily. If at any time the temperature is not within acceptable range then maintenance must be notified and a corrective action initiated. If the cooler is outside limits then the samples must be taken to another cooler for storage. If the room temperature is outside limits then no analysis is permitted. Document the corrective action number on the correct temperature log.

Note: On weekends, when no chemists are available, or when the office is closed- record TWR (temperature within range) as long as maintenance does not report that a temperature violation occurred.

XVII. Pipette Accuracy

Pipettes will be certified on a yearly basis with the certificate stating “as found” and “as returned”. If the pipette was found to be outside acceptable limits “as found” then a corrective action must be initiated.

XVIII. Forms

This section outlines the forms referenced in the earlier sections of this SOP. Please refer to the appropriate section to make sure the correct form is used. Forms will be reviewed annually for needed updates.

Form Name:

Form #:

a. Analyst Training Certification Checklist	PHL-13-01
b. Attestation Statement	PHL-13-02
c. Blood Alcohol Report Form	PHL-13-03
d. Blood Sample Log	PHL-13-04
e. GC Solution Prep and Analysis	PHL-13-05
f. Instructions for Blood Alcohol Sample Kit	PHL-13-06
g. Instructions for Urine Alcohol Sample Kit	PHL-13-35
h. Traffic/ Postmortem Blood Alcohol Sample Collection Form	PHL-13-07
i. Quality Control- Blood Sample Analysis Log	PHL-13-08
j. Quality Control Log for Standard Analyses	PHL-13-09
k. Sample Rejection Memo	PHL-13-10
l. Tube Labeling Reminder Memo	PHL-13-11
m. Competency Assessment Template	PHL-12-100
n. GC Tank Change/ Leak Check	PHL-13-31
o. Temperature Log	PHL-13-30
p. In-House PT Results Template	PHL-13-33
q. Emergency Instrument On/Off Procedure	PHL-13-34
r. Equipment Log	PHL-12-113
s. Thermometer Verification Data	PHL-12-117
t. AR-PHL Training Documentation	PHL-12-26
u. Disinfection Log	PHL-13-36