BLOOD ALCOHOL ANALYTICAL METHODS

Table of Contents

Revision History

Revision #	Description of Changes
1	Original issue, BLAC analytical methods were consolidated into a single method. Changes were made to: AM#1 sections 3.3.3.1, 4.1.3.1.2, 4.2.1.6, 4.2.2.2, 4.2.2.4, 4.3.6, 4.4.9, 4.5.1.1, AM#2 section 2.8.4.3.1, AM #3 section 3.3.2.2, AM #4 sections 4.5.1.1, 4.5.1.1.1, AM #5 section 5.3.2, 5.3.3.1. The following sections were added: AM #1 section 4.2.2.4.2, 2.9 and AM #3 section 3.3.2.6-8. Previous revisions and revision section numbering may not correspond to the current method. Refer to the archived method for the sections in place at the time of edit for section specific edits.
2	Changes were made to AM#1 Sections 4.2.2.4.2, 4.4.1.2, 4.4.5.3. AM#1 sections 4.4.7.1 and 2 were added. AM#2 Sections 4.1.2.1, 4.1.2.2, 4.2.1.3, 4.3.1. AM #2 section 4.1.2.3 was added. AM #2 sections 4.1.4, and 4.2.1.5 were deleted. AM#4: 1.1, 3.1.2, 4.1.1.5
3	Changes were made to AM #1 section 3.3.3.2, 3.6.2, 4.2.2.4.2, 4.3.6.1, 4.4.7.1 and NOTES were added. Sections 3.7, 3.6.2.1 1-2 and 4.4.5.4 were added. Changes were made to AM #4 in its entirety. AM #6 changes were made to section 4.2 and section 4.4 was added.
4	AM #6: changes were made to section 1.1. Sections 4.3 and 4.3.1 were deleted. Am #7: Changes were made to sections 4.2.1.1, 4.2.2.3, and 4.2.2.4.
5	AM#1: Added sections 3.1.1.1, 3.1.2.1, 3.1.7, 3.6.2.1, 3.6.2.2, 4.2.2.3.11, 4.3.8.2.1. Changes made to 3.1.5, 3.6.2, 3.7, 4.1.1.1.4, 4.1.2.4, 4.2.4.4, 4.3.3, 4.3.6.1, 4.4.5.3.1. AM#2: Added 4.1.2.4. Changes made to 4.1.2. AM#4: Changes made to 1.1, 4.1.1.3, 4.1.1.4, 4.1.2.1-4, 4.1.3 (entire section), 4.2.1. AM#6: changes to 4.4.1.
6	AM #1 Edits made to Section 1.1, added sections 4.2.2.4.3, 4.4.3.5, and 4.2.1.12. AM#2 added section 4.1.1.1.1
7	AM #1 Edits made to section 4.2.2.4.3, Section 4.4.3.5, 4.4.5.5 and 4.4.7.1.1 were added. AM #2 section 4.1.3.4.1 was added.
8	AM#1 2.1, 3.1, 4.4.3.1, 4.4.7.1.1 AM#2 3.0, 4.1.2, 4.1.2.4, 4.1.3 AM#3 4.1.5 AM#7 1.2
9	Changes to AM#1 4.2.1.6, 4.6.2 and added section 4.3.9 AM#6 4.4.1, 4.4.2
10	Edits made to AM#1 section 4.3.9.1 – 4.3.9.1.4, and 4.4.7.1
11	Edits made to AM#1 section 3.6.2.2, 4.2.2.3.8 and 4.3.9.1.3. Added sections 3.7.1-2 Edited AM#4 sections 4.1.1.3-4.

1.0 Background/References

1.1 Humans have consumed fermented beverages such as beer and wine since prehistoric times. Ethanol abuse is often manifest in driving under the influence (DUI), a problem that plagues every country. The National Highway Traffic Safety Administration (NHTSA) reported that the proportion of drivers involved in fatal crashes that had a BAC of 0.08 or above decreased from 35% in 1982 to 20% in 1997 and leveled off thereafter. Changes in alcohol laws and demographic changes are thought to be responsible for this positive trend. 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. Chronic excessive ingestion of ethanol is directly associated with serious neurologic and mental disorders such as brain damage, memory loss, sleep disturbances and psychoses. Alcohol is also involved in a high percentage of domestic disputes, many of which result in injury and/or death.

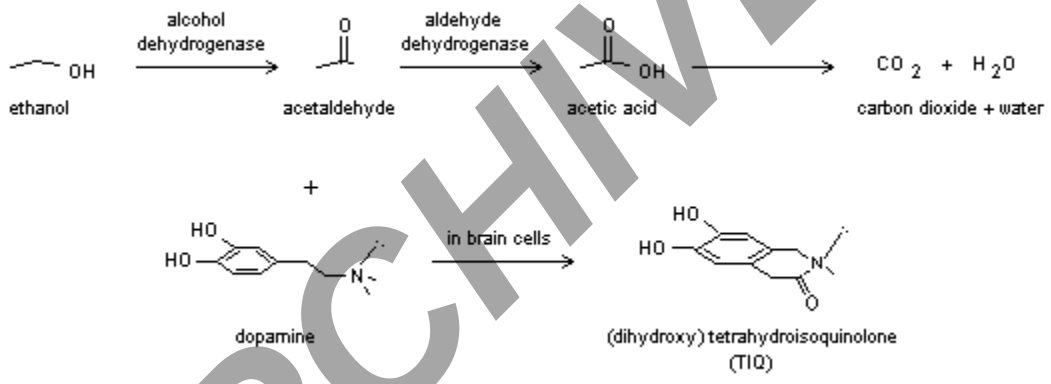
Notwithstanding a common public perception that ethanol is stimulatory, ethanol is classified as a *Central Nervous System Depressant.* Ethanol is a psychoactive drug that is similar in most respects to sedative-hypnotic compounds. The first mental processes to be affected are those that depend on training and previous experience. 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. The effects of ethanol and other central nervous system depressants are additive, resulting in more sedation and a greater degree of impairment in driving ability.

Ethanol is rapidly and completely absorbed from the stomach, small intestine and colon. The mechanism of absorption is a simple diffusion process; alcohol moves from a region of higher concentration to a region of lower concentration. Alcohol is soluble in both water and fat, a property that facilitates its diffusion through biological membranes. 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 vary greatly, depending upon a myriad of different factors ranging from the individuals stomach contents, the type of beverage consumed, the alcohol content, the rate of consumption, any disease state that affects normal gastric function or blood flow, any prescription or other drug therapy and many more unmentioned factors. Alcohol absorption is slowed by factors that delay gastric emptying. The time period in which a portion of the consumed alcohol remains in the stomach is a prime factor that contributes to the wide variety of absorption rates of ingested ethanol observed in different individuals and under different conditions. 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 contact with the blood supply.

Upon absorption, ethanol is distributed to all the water containing regions of the body. Within the blood system, there can be significant differences between arterial and venous blood depending upon the absorption status of the individual. 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 arterial and venous blood.

Ninety to ninety-eight 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 on to 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. In addition to gender, chronic abuse, prescription drugs and certain genetic factors can also influence the elimination rate.

Figure 1. Metabolism of Ethanol.



Other commonly encountered toxic alcohols, alcohols other than ethanol that are not intended to be ingested, such as methanol and isopropanol, produce central nervous system (CNS) depressant effects but vary significantly in the degree. Methanol (wood alcohol), commonly a component of model airplane fuel and windshield wiper fluid, causes relatively little intoxication compared to ethanol. Its harmful effects 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. The formic acid leads to metabolic acidosis. Isopropanol (rubbing alcohol) is more toxic than ethanol with more prominent gastritis that includes pain, nausea, vomiting and hemorrhage. Isopropanol is metabolized to acetone. Note that elevated endogenous acetone may also be detected in the samples from a diabetic or fasting individual.

A variety of volatile chemicals may also be detected in samples from subjects that were inhaled either accidentally or intentionally. For instance, toluene and acetone may be detected in subjects that come into contact with products such as aerosol paint and contact adhesives. The principal metabolite of toluene is benzoic acid. Chronic abuse of toluene and/or acetone can lead to organ and CNS problems that may result in permanent damage. Acetone is metabolized to acetate and formate. Inhaling of electronic cleaning products referred to as computer "dusters" can result in detectable levels of the aerosol propellants 1,1-Difluoroethane (HFC-152a) and 1,1,1,2-Tetrafluoroethane (HFC-134a). The primary consequence of abuse is cardiovascular in nature.

The analysis of ethanol and other volatiles in samples of blood, vitreous humor and urine is accomplished with a gas chromatograph (GC) which uses a headspace autosampler (HSA) for sample introduction. An aliquot of sample is placed into a headspace vial along with an aqueous 1-propanol internal standard in 1M Ammonium Sulfate. The sample vials are sealed and heated in a HSA. 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. The ammonium sulfate serves as a salting- out agent, thus improving the recovery of volatiles from the headspace. An aliquot of the headspace vapor is injected into a GC with a dual column configuration. The GC serves to separate out the components of the solution as a function of their chemical properties. Separated components are detected by a flame ionization detector (FID). The qualitative identification of ethanol and other common volatiles is based on the retention time determined, relative to the 1-propanol internal standard, for each of the columns. This method also provides for a quantitative determination for ethanol. The quantitative result is based on a calibration curve established by the peak area ratio between ethanol and the 1-propanol internal standard.

The need to establish the ethyl alcohol concentration and/or the presence of other commonly encountered volatiles in a beverage or solution may arise from ABC violations (Idaho Code 23-611, 23-1002, 23-1303, ...), under-age consumption (Idaho Code 23-603, 23-604), open-container violations (Idaho Code 23-505, 23-1333), poisonings and/or an endless variety of situations including questionable samples submitted as blood or other physiological fluid. In addition, ethyl alcohol concentration must be verified in simulator solutions used for breath testing instruments (IDAPA 11.03.01).

1.2 References

- Stafford, D.T., *Chromatography. in:* Principles of Forensic Toxicology, edited by Barry Levine, pp. 91-98, 100-108, 114-118, AACC Press, 2006.
- Levine, B. and Caplan, Y.H., *Alcohol*. *in:* Principles of Forensic Toxicology, edited by Barry Levine, pp. 169-184, AACC Press, 2006.
- Caplan, Y.H., *The Determination of Alcohol in Blood and Breath. in:* Forensic Science Handbook, edited by Richard Saferstein, pp. 594-648, Prentice-Hall New Jersey, 1982.
- Julien, R.M., Central Nervous System Depressants: Alcohol and the Inhalants of Abuse. in: Primer of Drug Action, pp. 64-92, Freeman-New York, 1998.
- Saker, E.G., Screening and Quantitation by Head Space Technique of Some of the Vapors Most Commonly Found in Forensic Toxicology, in: Current Approaches in Forensic Toxicology, Chapter 11, SOFT Meeting, 1994.
- Perrine, D.M., *Depressants: Alcohol, Benzodiazepines, Barbiturates*. *in:* The Chemistry of Mind-Altering Drugs, pp. 113-129, ACS, Washington, DC, 1996.
- Hobbs, W.R., Rall, T.W. and Verdoorn, T.A., *Drugs Acting on the Central Nervous System - Hypnotics and Sedatives; Ethanol. in:* Goodman and Gilman's The Pharmacological Basis of Therapeutics, pp. 361, 386-393, McGraw-Hill, 1996.
- Idaho Administration Code, IDAPA 11.03.01, Rules Governing Alcohol Testing.
- Jones, A.W., *Disposition and Fate of Ethanol in the Body. in:* Medical-Legal Aspects of Alcohol, edited by J.C. Garriott, pp. 87 - 90, Lawyers & Judges Publishing Co., Inc., 2003.
- Caplan, Y.H., Goldberger, B.A., *Blood, Urine and Other Fluid and Tissue Specimens for Alcohol Analyses. in:* Medical-Legal Aspects of Alcohol, edited by J.C. Garriott, pp. 152-153, Lawyers & Judges Publishing Co., Inc., 2003.
- Anderson, W.H, *Collection and Storage of Specimens for Alcohol Analysis. in:* Medical-Legal Aspects of Alcohol, edited by J.C. Garriott, pp. 241-245, Lawyers & Judges Publishing Co., Inc., 2003.
- 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.
- 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.
- Klaassen, C.D., *Nonmetallic Environmental Toxicants. in:* Goodman and Gilman's The Pharmacological Basis of Therapeutics, pp. 1681-1682, McGraw-Hill, 1996.

- Broussard, L.A., *Inhalants. in:* Principles of Forensic Toxicology, edited by Barry Levin, pp. 373-380, AACC Press, 2006.
- Baselt, R.C., *Ethanol*. *in*: Disposition of Toxic Drugs and Chemicals in Man, pp. 411-414, Biomedical Publications, 2004.
- Dang, J.N., Statistical Analysis of Alcohol-related Driving Trends, 1982 - 2005, NHTSA Technical Report No. DOT HS 810 942, May 2008.
- Wiener, S.W., *Toxic Alcohols*, *in*: Goldfranks's Toxicologic Emergencies, pp. 1447 - 1457, McGraw-Hill, 2006.
- Pounder, D.J. and Jones, A.W., *Measuring Alcohol Postmortem*, *in*: Drug Abuse Handbook, edited by Steven Karch, pp. 376-389, McGraw-Hill, 2007. Shaw, R.F., *Methods for Fluid Analysis. in:* Medical-Legal Aspects of Alcohol, edited by J.C. Garriott, pp. 220-222, Lawyers & Judges Publishing Co., Inc., 2003.
- Avella, J., Lehrer, M., Zito, S.W., A Validated Method for the Quantitation of 1, 1-Difluoroethane Using a Gas in Equilibrium Method of Calibration, J. Anal. Toxicol. **32(8):** 680-687 (2008).

2.0 Scope

2.1 This method describes the Idaho State Police Forensic Services (ISPFS) procedure for the analysis of blood, vitreous humor, urine and solutions for the presence of volatile compounds. This method provides for the quantitative analysis of ethanol as well as the qualitative analysis of methanol, acetaldehyde, acetone, isopropanol, toluene, fluorinated hydrocarbons and related compounds, via a headspace sampling gas chromatographic method. The words calibrator and calibration are used to coincide with the terminology in instrument software and manufacturer manuals. The manufacturer's term calibrator refers to what is considered by ISP-FS as aqueous certified reference material (CRM) that has a certified concentration of ethanol present. This aqueous reference material is used to establish a calibration curve/table to establish a response factor between instrument response and reference material concentration.

2.2 If this method is applied specifically for the qualitative identification of volatiles other than ethanol, ethanol calibrators and controls need not be included in the analysis run.

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 Agilent 7890A Gas Chromatograph (GC), Shimadzu Nexis GC-2030AF or equivalent, configured with Flame Ionization Detectors (FIDs)

3.1.1.1 Instrument parameters: Quantitative method only

Oven temperature: set at or above 30°C

- If utilizing a temperature ramp, must not exceed 10°C/min

Inlet temperature: set at or above 100°C

Detector temperature: set at or above 220°C

3.1.2 Agilent G1888, Agilent 7697, Shimadzu HS-20 or equivalent Headspace Sampler

3.1.2.1 Recommended instrument parameters: Quantitative method only

Oven temperature: set at or above 40°C isothermal

Loop temperature: set at or above 100°C

Transfer line temperature: set 10°C above loop temperature (if applicable)

3.1.3 Columns

3.1.3.1 Restek Rtx-BAC1, SH-RTX-BAC Plus 1 (30 meter X 0.32mm inner diameter (ID), 1.8µm film thickness (FT) or equivalent column)

3.1.3.2 Restek Rtx-BAC2, SH-RTX-BAC Plus 2 (30 meter X 0.32mm ID, 1.2 µm or 0.6 µm FT or equivalent column)

3.1.4 Headspace (HS) vials and Closures

3.1.5 Hand Crimper or Bench Top Crimper (only needed for crimp top vials)

3.1.6 Semi-Automatic Dilutor/Pipettor equipped with sample and reagent syringes capable of dispensing 250µL and 2000µL, respectively

3.1.7 Tissue grinder, or equivalent liquid homogenizer.

3.2 Reagents

3.2.1 Distilled/Deionized water (free from volatiles of interest)

3.2.2 Ammonium Sulfate (Certified ACS Grade)

3.3 Reference Material

3.3.1 Ethanol Aqueous Reference Material

3.3.1.1 Aqueous ethanol reference material used to establish the calibration curve/table or to prepare aqueous ethanol controls can be obtained through a commercial vendor. *Aqueous reference material used to establish the calibration curve must be traceable to NIST standards.*

3.3.2 Multicomponent Volatile Aqueous Solutions

Multicomponent solutions may be purchased or prepared as indicated below.

3.3.2.1 Commercially Obtained Multicomponent Solution

3.3.2.1.1 Solution may include acetone, ethanol, methanol and isopropanol reference materials and/or commonly abused volatiles.

3.3.2.1.2 When the multicomponent solution contains quantitative amounts of ethanol, it may simultaneously serve as an aqueous ethanol control. The GC oven temperature program must provide for baseline separation of all components.

3.3.2.2 Prepared Mixed Volatile Reference Solution

3.3.2.2.1 Add approximately 250 mL of DI water to a flask. Add **one or more** of the following volatiles, as needed for the qualitative identification of volatiles:

Compound	Volume
Acetaldehyde	≅100µL
Acetone	≅100µL
Ethanol	≅100µL
Ethyl Acetate	≅100µL
Methanol	≅500µL
Isopropanol	≅500µL
Toluene	≅50µL

3.3.2.2.2 *Record preparation on reagent log.* Solution is stable indefinitely when stored under refrigeration.

3.3.2.2.3 Additional volatiles of interest may be used singularly or added to the mixed volatile solution.

3.3.2.2.4 The GC oven temperature conditions must provide for baseline separation for all components placed in the mixture.

3.3.2.3 Fluorinated Hydrocarbon Reference Solution

Fluorinated hydrocarbon reference solutions may be prepared from commercially obtained aerosol products. The product's MSDS should be obtained. Solutions may be used for as long as acceptable performance is obtained.

3.3.3 Internal Standard Solution (~0.03g/dL 1-propanol in 1.0M Ammonium Sulfate)

3.3.3.1 Add ~132g $(NH_4)_2SO_4$ per L of solution to be prepared and mix with DI water to dissolve. Add ~375µL 1-propanol per L of solution to be prepared. A maximum of 4L may be prepared at a time.

3.3.3.2 Record preparation and lot # of 1-propanol on reagent log. Solution is stable up to 6 months.

3.4 Matrix Control Material

Refer to Blood Alcohol AM# 2 for authentication requirements.

3.4.1 Ethanol or Multicomponent Whole Blood Control Material

3.4.2.1 A whole blood control containing ethanol with other volatile can serve as a multicomponent control if the GC oven temperature program provides for baseline separation of all components.

3.4.2.2 A minimum of two ethanol whole blood control levels should be available, each falling within the following approximate ranges:

Level	Approximate Ethanol Range (g/100mL)
Low	0.030 – 0.130
High	0.131 – 0.400

3.5 Safety Concerns

Biological samples must be processed, and chemicals handled according to safety guidelines in the *Idaho State Police Forensic Services Health and Safety Manual.*

3.6 Quality Assurance

3.6.1 While at the laboratory samples for volatiles testing are to be stored under refrigeration. Urine samples can be either refrigerated or frozen. Urine samples submitted in plastic bottles must be frozen for long-term storage.

3.6.2 The Pipette/Dilutor must be checked for precision. This check is monitored with the establishment of the calibration curve and the use of authenticated controls during an analysis run.

3.6.2.1 Once per calendar year, the pipette/dilutor will be calibrated by an approved vendor/service provider.

3.6.2.2 Intermediate checks will be performed after maintenance on the pipette/dilutor or after external calibration services. Calibration services performed on-site, within an ISPFS lab, do not require an intermediate check before being put back into use within the same laboratory.

3.6.3 Refer to Toxicology Analytical Method for balance intermediate check and calibration requirements.

Note: Balances properly monitored by drug discipline analysts fulfill quality assurance requirements. Additional check need not be performed.

3.6.4 Refer to manufacturer manuals for as-needed instrument maintenance procedures and troubleshooting measures.

3.6.5 Current source and lot number of controls and reference material must be documented.

3.6.6 Refer to Blood Alcohol AM# 2 for reference material authentication requirements.

3.6.7 Refer to Blood Alcohol AM# 7 for quantitative ethanol reporting confidence interval/uncertainty.

3.6.8 If ethanol is not the analyte in question, a calibration curve for ethanol need not be established.

3.7 Storage. All reference material should be stored in refrigerated conditions when not in use. This should be monitored and between **-2°C to 12°C**. Matrix Controls should be stored frozen, **below 0°C**.

3.7.1 Transportation for calibrators and controls between ISPFS labs should be done in a similar manner as case samples when shipped.

3.7.2 During general transport (non-shipped), controls should be stored refrigerated/cold or frozen and calibrators should be stored refrigerated/cold.

4.0 Procedure

4.1 Collection Kit Processing

4.1.1 Collection Kit Description and Labeling

4.1.1.1 Record the following information:

4.1.1.1.1 A description of collection kit type.

4.1.1.1.2 A description of type and number of specimen collection container(s).

4.1.1.1.3 If it is apparent that the specimen container does not appear to be the one originally included in collection kit.

4.1.1.1.4 The sample type (blood, urine, vitreous humor, other.

4.1.1.2 Laboratory number must be placed on each sample container.

4.1.1.3 When more than one sample is present, label all samples present. Use "A", "B", etc. or comparable in addition to the laboratory number.

4.1.2 Blood Specimen Collection Container Evaluation

4.1.2.1 Idaho Administrative Code, IDAPA 11.03.01 requires law enforcement agencies to have blood specimens, from living subjects, collected in a container containing *at least ten (10) milligrams of sodium fluoride per cubic centimeter of blood plus an appropriate anticoagulant.* [8]

4.1.2.2 The containers provided in ISPFS kits comply with IDAPA requirements. It must, however, not be assumed that an ISP-FS kit contains the specimen collection tubes it was supplied with.

4.1.2.3 Non-ISP-containers must be evaluated as to compliance. Information from the manufacturer of the container will indicate sodium fluoride concentration as well as the presence of an appropriate anticoagulant.

4.1.2.3.1 Note compliance of blood specimen container.

4.1.3 Blood Specimen Evaluation

4.1.3.1 IDAPA 11.03.01 requires blood to be reported as grams of alcohol per 100cc of whole blood. Although the absolute determination that the sample is whole blood is beyond the scope of this analytical method, when it is the analyst's opinion that the intended blood sample is serum or otherwise questionable, the analyst has the following options.

4.1.3.1.1 <u>Option One</u>

The sample is not analyzed. A comment "Specimen unsuitable for analysis" is placed on the analysis report.

4.1.3.1.2 Option Two

The sample is analyzed for volatiles, and the report will make no mention of the sample having a biological origin or contain the disclaimer that the "sample(s) appears to be (insert type)".

NOTE: For conversion purposes, the lab will use the range of 1.12 – 1.17 for the conversion of serum alcohol results to whole blood alcohol results.

4.2 Analysis Procedure

4.2.1 Calibration Curve/Table Requirements

4.2.1.1 A minimum of three ethanol aqueous reference solutions must be used to establish calibration/response factor curve.

4.2.1.2 The minimum low calibrator is to be in the nominal range of approximately 0.02 to 0.05g/100mL.

4.2.1.3 The highest calibrator concentration must be 0.30g/100mL or greater.

4.2.1.4 Calibration table may be established in a separate batch just prior to the batch containing case samples.

4.2.1.5 Ethanol calibrators should be analyzed in order of increasing concentration, and after being diluted with internal standard should be used for the generation of only one calibration curve. Calibrators should not be saved and used to generate future curves. The manufacturer's target value is defined as the manufacturer's "as prepared" certified concentration, and not the "as analyzed" value.

4.2.1.6 The R^2 value is to be used for the evaluation of the linearity of the curve data in the GC-HS Software. The R^2 resulting from the analysis of the ethanol calibrators must have a value of ≥ 0.999.

4.2.1.7 Each ethanol calibrator may have more than one replicate.

4.2.1.7.1 In the batch table, on the Update RF column, select "replace" for each of the first set of calibrators. If a second set of calibrators is run and are to be included in the generation of the calibration curve, the results are to be "averaged". (Software version may differ slightly)

4.2.1.8 If data from a calibrator is not usable, the remaining data can be used to establish the response factor if requirements in 4.2.1.1, 4.2.1.2, 4.2.1.3 and 4.2.1.6 have been met.

4.2.1.9 A calibration curve/table is valid for 14 days, provided:

Values for required controls fall within acceptable ranges.

The same preparation of internal standard solution used for the calibration run is used.

4.2.1.10 Once established, analysts not involved in establishing the calibration curve/table may use the established calibration table.

4.2.1.11 An internal standard blank should immediately follow the highest ethanol calibrator.

4.2.1.12 Refer to BrAC AM#1 section 4.0 for the criteria for running samples for the purpose of authenticating breath alcohol simulator solutions.

4.2.2 Analysis Run Control and Blank Requirements

4.2.2.1 Initial Run with Calibration Curve

For a run with a newly established calibration curve, an ethanol containing control must precede the first 10 samples (20 vials). The control must be run in duplicate. An additional control must be run at the end of the quantitative samples being analyzed so that the samples are bracketed by ethanol containing control samples.

4.2.2.2 Additional Runs with Existing Calibration Curve

For analysis runs utilizing an existing calibration curve, a low and high ethanol-containing control, in duplicate, must bracket the first 10 samples (20 vials) before proceeding with additional samples. Each additional set of 10 samples (or less) must also be bracketed by an ethanol containing control.

4.2.2.2.1 Samples run for qualitative purposes are not required to be bracketed by controls and can be added to the end of an existing set of samples.

4.2.2.3 All Analysis Runs

4.2.2.3.1 Each analysis run must contain an internal standard blank.

4.2.2.3.2 For analysis runs consisting of more than 10 case samples (20 vials), a minimum of one blood or aqueous ethanol-containing control in duplicate must be run with each additional 10 samples. Each set of 10 (or less) quantitative case samples must be bracketed with ethanol containing controls.

4.2.2.3.3 Each analysis run must include either an *aqueous* or *blood* multicomponent volatile mix.

4.2.2.3.4 A commercially obtained **quantitative** *multicomponent* volatile mix may serve as both an aqueous *ethanol control* and a *multicomponent mixture*.

4.2.2.3.5 Each run must contain a blood matrix control in duplicate, as defined in Blood Alcohol AM# 1, section 3.4.2.1.

4.2.2.3.6 Each run, new or previously calibrated, must contain a traceable aqueous control in duplicate at or near the 0.080 level for control charting purposes and for an accuracy QC check. This shall be a newly opened ampoule and will be used to monitor the accuracy of the method and instrumentation over time. This sample is run as if it were a case sample and counts as if it were a case sample for control-bracketing purposes. The aqueous control sample must be bracketed by ethanol containing controls as if it were a regular quantitative case sample.

NOTE: If an analyst utilizes a previous calibration curve generated by a different analyst, then the analyst that generated the original calibration curve cannot participate in the review of the new casework.

4.2.2.3.7 Additional aqueous controls may be run at the end of the batch to monitor the overall performance of the instrument, but does not need to meet the acceptance criteria set in 4.3.

4.2.2.3.8 For controls run in duplicate utilizing the GC-HS software, the samples should contain the designator used by the software macro for generation of the data sheet and ease of evaluation. Otherwise, the data must be put into the data sheet manually for evaluation purposes.

4.2.2.3.9 Controls of the same lot number (either aqueous or matrix control), shall not be used for multiple (different) purposes within the same batch. (i.e. The 0.080 aqueous control cannot be used as both an aqueous run control and the control charting control during the same batch).

4.2.2.3.10 Infrequently run case samples cannot be included as part of a previously calibrated run. The infrequently run case samples must be run as part of a batch utilizing a new calibration curve.

4.2.2.3.11 Quantitative Beverage Sample cases are currently the only infrequently run type of case.

4.2.2.4 Qualitative Only Analysis Runs

Sections 4.2.1, and 4.2.2.1 - 4.2.2.3.9 do not apply for an analysis run consisting of only qualitative samples.

4.2.2.4.1 Runs consisting of only qualitative samples need only the sample in duplicate, and the volatile reference standard in question separated by an internal standard blank at a minimum.

4.2.2.4.2 To qualitatively identify a peak in a sample that was run as part of a regular batch for alcohol, the analyst needs only to run the qualitative confirmation standard mixed with internal standard of the same chemical component as the original sample run for comparison purposes. The qualitative standard should be run within 72 hours of the sample in question.

4.2.2.4.3 For unknown analytes of interest, analysis may be conducted on the sample utilizing structural determinations methods and instrumentation (GC/MSD, LC/MS, etc). Structural analysis needs to be performed by individuals qualified to use the required instrumentation.

4.2.2.5 Aqueous Controls

Lots used in the establishment of the calibration curve must not be used as aqueous controls during a run using said calibration curve.

4.2.3 Sample Preparation

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

4.2.3.2 Sample preparation must take place in a laminar flow hood or biological safety cabinet.

4.2.3.3 Place blood sample container on rocker to mix the sample contents.

4.2.3.4 If a blood sample appears to be coagulated, the sample may require homogenization in a tissue grinder, or equivalent.

4.2.3.5 All case samples must be analyzed in duplicate.

4.2.3.6 Use Pipette/Dilutor to dispense 250µL of case sample, positive control, or calibrator solution, along with 2000µL of internal standard (ISTD), into labeled headspace vial and apply seal.

4.2.3.7 For internal standard blank, dispense 250µL of DI water along with 2000µL of internal standard (ISTD) into labeled headspace vial and apply seal.

4.2.3.8 Dilute alcoholic beverages and unknown solutions as necessary. The sample must be diluted for the value to fall within the upper limits of the calibration curve. Generally, beer and wine should be diluted ~50:1 with DI water and distilled beverages (≥ 16% w/v or 20% v/v) diluted ~100:1. If available, the dilution of unknown solutions should be based on sample history.

4.2.3.8.1 Dilution may be carried out using the autodilutor. If the autodilutor is used, the uncertainty of measurement must reflect the correct number of uses of the autodilutor in the final calculation.

EXCEPTION: if the agency requests qualitative analysis only for the ethanol content of an unknown liquid, then the sample need not fall within the limits of the calibration curve as long as the peak (shape, width, height) is acceptable and within the retention time acceptance criteria.

4.2.3.9 Breath testing simulator solutions and samples, which appear to be serum, do not require pre-dilution.

4.2.4 Instrument Run Preparation

4.2.4.1 Open **Batch Table.** It is recommended that each analyst create, not share, a Batch Table. This reduces the possibility of the Batch Table being modified without their knowledge. If a Batch Table is shared, each analyst must inspect the Batch prior to analysis.

4.2.4.2 In the Batch log table, enter the sample case numbers, ethanol calibrators, volatiles single constituent reference material, volatile reference material mixtures, blank(s) and controls.

4.2.4.3 Load samples, calibrators, blank(s), reference material(s) and controls onto the headspace sampler rack as noted in the batch table.

4.2.4.4 The batch information should be checked prior to starting the instrument.

4.2.5 Instrument Parameters

4.2.5.1 Refer to current instrument method for gas chromatograph and headspace analyzer analysis parameters.

4.2.5.2 Analysis method must be stored centrally (hardcopy and/or electronically) each time the method parameters are updated.

4.3 Criteria for Acceptance of Data

4.3.1 All sample and control values must have a calibrator greater than or equal to their mean value.

4.3.2 For samples above the highest calibrator used to establish calibration curve/table, the sample must be reanalyzed after dilution. The dilution factor is incorporated into final calculations.

4.3.3 All results obtained from samples bracketed by conforming controls are acceptable for use, provided the chromatography is acceptable, retention times are correct and the internal standard is present.

4.3.4 When the overall mean of a control value falls outside of required qualitative, quantitative and/or precision acceptance criteria, the 10 casework samples preceding and following the non-conforming control(s) must be reanalyzed. If only the quantitative criteria are not met, this reanalysis requirement does not apply to samples that are being processed for the qualitative presence of volatiles other than ethanol.

4.3.5 Qualitative Accuracy Criteria

The qualitative presence of ethanol, or other volatile substances, can be established if the retention time for a specimen is within ±0.10 minutes of the retention time of the reference compound in question. This criterion should be designated in the instrument's data station analysis method.

4.3.6 Quantitative Accuracy Criteria

The quantitative ethanol results for a batch of samples can be accepted if the values obtained for control samples fall within ±10% of the established target value.

4.3.6.1 The acceptability criteria for the aqueous control from Blood Alcohol AM# 1 section 4.2.2.3.6, will be that the overall reported value (as if it were a casework sample) must fall within the currently accepted level for the process uncertainty of measurement, as established by Blood Alcohol AM# 7, of the target value.

4.3.7 Column Precision Criteria

4.3.7.1 The ethanol values obtained from column 1 and 2 must agree within 0.0100g/100cc (exclusive of post mortem samples).

4.3.7.1.1 For postmortem samples, if the sample fails to meet the criteria in 4.3.7.1, the analyst shall report the lowest single column result average.

4.3.7.2 If the precision requirement is not met, the sample must be reanalyzed. If upon reanalysis, the column precision requirement is not met, the source of the problem will be pursued. One possible cause is a system leak.

4.3.8 Quantitative Replicate Precision Criteria

4.3.8.1 The mean value for replicate analysis must agree as described in the following table. If the precision requirement is not met, the sample must be reanalyzed.

Results Range (g/100cc)	Precision (g/100cc)
0.0200 - 0.1099	0.0100
0.1100 - 0.2299	0.0150
0.2300 - 0.3499	0.0200
0.3500 - 0.5000	0.0300

4.3.8.2 If upon re-analysis, the replicate precision requirement for control sample(s) is not met, troubleshooting must be initiated and documented. Case samples may require additional homogenization.

4.3.8.2.1 Post-mortem samples not meeting the replicate precision criteria after reanalysis, can be reported out as the average of the lower pair of results.

4.3.8.3 If desired, a macro can be created and installed on the instrument to display the analysis data and populate it to a form/spreadsheet.

4.3.9 Internal Standard Recovery Monitoring

4.3.9.1 The internal standard values within the samples will be monitored and recorded to evaluate the quality and consistency of injection for each sample within the run.

4.3.9.1.1 The average values for the internal standard will be established by averaging the IS counts for FID1 and FID2 throughout the matrix and aqueous quality control samples used throughout the run.

- The QC1(s), QC2(s) and 0.080 aqueous control samples will be used to monitor injections. The internal standards are only present to demonstrate the lack of sample carryover and internal standard performance.
- Average values will be established for the Internal standard response for both FID1 and FID2.

4.3.9.1.2 Those values will establish the mean recovery value.

4.3.9.1.3 Acceptable IS recovery values for case samples run with a specific calibration curve must have their FID1 and FID2 IS values fall within +/- 20% of the mean values established in 4.3.9.1.1.

4.3.9.1.4 Samples with ISTD values that fall outside of this established range must be rerun using a new vial pair (vials A and B must be re-prepared and rerun).

4.4 Reporting of Results

4.4.1 General

4.4.1.1 The three decimal place truncated mean ethanol value, as determined by this method, will be reported along with the confidence interval range in the form of the uncertainty of measurement (as established by section 7.0).

4.4.1.2 The uncertainty of measurement shall be reported out to three decimal places rounded up (+/- X.XXX)

4.4.2 Blood Ethanol Results

4.4.2.1 Report overall mean ethanol concentration, as grams of ethanol per 100cc of blood, truncated to three decimal places ± the uncertainty of measurement.

4.4.2.2 Report values <0.020g/100cc, but above 0.000 as "*below reportable limit.*". Results that are 0.000 shall be reported as "none detected".

4.4.2.3 If the sample and/or sample vial clearly does not comply with IDAPA 11.03.01, an appropriate comment must be noted on the analysis report.

4.4.3 Urine Ethanol Results

4.4.3.1 The four decimal place overall mean ethanol value must first be multiplied by 0.67, unless results are measured at below 0.020 g/100cc.

4.4.3.2 Report overall mean ethanol value as grams of ethanol per 67mL of urine truncated to three decimal places ± uncertainty of measurement.

4.4.3.3 Report values <0.020g/67ml, but above 0.000 as "*below reportable limit*". Results that are 0.000 shall be reported as "none detected".

4.4.3.4 A qualifier statement "*Urine results may be of questionable value*" **must** be included in the analysis report for ethyl alcohol determination. The disclaimer is not required for other volatiles reported qualitatively.

4.4.3.5 Results that fall in the ranges outlined in 4.4.3.3 do not need to have uncertainty of measurement calculations performed on casefile worksheets.

4.4.4 Vitreous Humor Ethanol

4.4.4.1 Report overall mean ethanol concentration, as grams of ethanol per 100cc of vitreous humor, truncated to three decimal places (0.000) ± uncertainty of measurement.

4.4.4.2 Report values <0.020g/100cc, but above 0.000 as "*below reportable limit.*". Results that are 0.000 shall be reported as "none detected".

4.4.4.3 No conversion to a blood alcohol value will be made on the report.

4.4.5 Alcohol Beverages

4.4.5.1 To obtain the ethanol concentration value, the overall mean ethanol concentration results are multiplied by the dilution factor (if applicable). This will provide the ethanol concentration in g/100cc (weight per volume (w/v) percent).

4.4.5.2 For volume per volume (v/v) value, divide w/v value by 0.79.

4.4.5.3 Value must be reported as both w/v and v/v percent. The mean value must be truncated and reported out to the tenths decimal place ± the uncertainty of measurement, rounded up to the tenths decimal place (+/- XX.X).

4.4.5.3.1 At the request of the submitting agency, beverage samples being run qualitatively may be reported out at positive or negative for the presence of ethyl or other volatiles/alcohols. Report wording should be consistent with qualitative analysis reporting criteria.

4.4.5.4 At the discretion of the analyst, beverages samples may be treated as unknown liquids if their alcohol results fall below 1.0 and would be reported as less than a whole number.

4.4.5.5 If the results of the sample, after dilution and analysis, are still above the range of the calibration curve, then the sample may be reported as "above reportable limit". Cases of this nature are of such extreme concentration that column precision and replicate requirements are not required in order to report the results.

4.4.6 Unknown Liquids and "Serum" - Ethanol

4.4.6.1 Report ethanol concentration in g/100cc and/or volume per volume (v/v) percent, depending on the sample history.

4.4.6.2 When dilution is necessary, the overall mean results of analysis must be multiplied by the dilution factor.

4.4.6.3 When reporting as g/100cc, report overall mean ethanol concentration, truncated to three decimal places (X.XXX), as grams of ethanol per 100cc of liquid ± the uncertainty of measurement.

4.4.7 Reporting of Qualitative Volatiles Results

4.4.7.1 Reporting qualitative volatiles is at the discretion of the analyst. If reported, other volatiles such as acetone, isopropyl alcohol, methyl alcohol, toluene and formaldehyde will be noted on the analysis report following the ethyl alcohol results.

4.4.7.1.1 When reporting acetone, the statement "Acetone could be produced in the body as a metabolic by-product of diet or disease." should be added to the report.

4.4.7.2 The qualitative presence of fluorinated hydrocarbons, such as Difluoroethane, will be reported out as containing "fluorinated hydrocarbons". Further explanation of "fluorinated hydrocarbons" is at the discretion of the analyst.

4.4.8 Comments for Analysis Report

As appropriate and/or required, comments outlining actions, discrepancies and/or qualifiers can be included on the analysis report following the results of analysis.

4.4.9 Breath Testing Solutions

Provide results to Discipline Leader for evaluation.

4.4.10 New Blood Matrix Control Evaluation

Provide results to Discipline Leader for evaluation.

4.5 Analysis Documentation

4.5.1 Volatiles Analysis Forms

4.5.1.1 Spreadsheet form for calibrator, controls and case sample can be located on the ISP Qualtrax system.

4.5.2 Quality Assurance Data

4.5.2.1 A copy of quality assurance data (calibrators and controls) need not be included in individual case files.

4.5.2.2 A packet containing spreadsheets and data for response factor/calibration curve, controls and reference material will be prepared for each analysis run and stored centrally in the location designated for alcohol quality assurance data in the laboratory where the analysis was performed until archiving.

4.5.2.2.1 The storage of central data may be done electronically.

4.5.2.3 When necessary, a copy of the quality assurance data can be prepared from the centrally stored documents or reprinted from electronically stored data.

4.5.2.4 For qualitative only runs, the only QA samples that need to be included are the qualitative reference standard used to identify the peaks of interest and the appropriate blanks (internal standard and optional water blank).

4.6 Maintenance

4.6.1 Consult the instruments maintenance manual for types of maintenance available for this instrument.

4.6.2 A cleaning or "baking" method be should developed in each laboratory that is specific for the instrument. The baking procedure should increase the temperatures of the samples pathway through the instrument.

4.6.2.1 The cleaning method is to be run on an "as needed" basis that is determined by the analyst. The indicators include but are not limited to, changes in the peak shape, baseline noise, column precision, and retention time drift.

4.6.2.1.1 G1888 headspace/7890A GC parameters example:

- HS Oven Temp: 100ºC
- Loop Temp: 200ºC
- Transfer Line Temp: 200ºC
- Inlet Temp: 220ºC (for BAC1/BAC2 columns)
- GC Oven Temp: 220ºC (for BAC1/BAC2 columns)
- Detector Temp: 250ºC

4.6.3 On a yearly basis, all of the data files and calibration curve data will be backed up to permanent media, or equivalent backup device.

Analytical Method #2: Authentication

1.0 Background/References

1.1 Refer to Blood Alcohol AM#1.0, section 1.1

1.2 References:

- Stafford, D.T., *Chromatography. in:* Principles of Forensic Toxicology, edited by Barry Levine, pp. 91-98, 100-108, 114-118, AACC Press, 2006.
- Levine, B. and Caplan, Y.H., *Alcohol in:* Principles of Forensic Toxicology, edited by Barry Levine, pp. 169-184, AACC Press, 2006.
- Caplan, Y.H., *The Determination of Alcohol in Blood and Breath in:* Forensic Science Handbook, edited by Richard Saferstein, pp. 594-648, Prentice-Hall New Jersey, 1982.
- 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.
- 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.

2.0 Scope

2.1 This method describes the Idaho State Police Forensic Services (ISPFS) requirements for the authentication of quality assurance material used to provide confidence in the data collected during the analysis of blood, vitreous humor and urine to establish both the qualitative and quantitative presence of ethanol and other volatiles.

3.0 Equipment/Reagents

3.1 Equipment: Refer to Blood Alcohol Analytical Method #1, section 3

3.2 Reagents: Refer to Blood Alcohol Analytical Method #1, section 3.2

3.3 Reference Material: Refer to Blood Alcohol Analytical Method #1, section 3.3

4.0 Procedure

4.1 Authentication of Volatiles Reference Materials

4.1.1 General

4.1.1.1 Refer to Blood Alcohol AM 1 for GC-HS analysis requirements.

4.1.1.1.1 Refer to BrAC AM#1 section 4.0 for the certification process of authentication breath alcohol simulator solutions.

4.1.1.2 **Aqueous** reference material used to establish the calibration curve must be traceable to NIST standards.

4.1.1.3 All available Certificates of Analysis for reference material will be stored centrally (either via hard copy or electronically).

4.1.1.5 Reference materials without certificates of analysis will be authenticated structurally.

4.1.1.4 New lots of reference material must be authenticated prior to an analyst reporting a conclusion in casework in which that reference material was used.

4.1.2 Authentication Process for reference material purchased from an approved 17025/17034 or equivalent provider.

4.1.2.1Quantitative reference materials must be purchased from an ISO 17025/17034 accredited supplier (Or equivalent) with analysis certification to within +/- 5% of the stated target value.

4.1.2.2 Reference Standards documentation and analysis criteria must be checked by any analyst prior to use. The manufacturer's "as prepared" target value will be used as the target value for the lot.

4.1.2.3 Reference material supplier's current accreditation documentation should be on file with the Quality Manager.

4.1.2.4 Qualitative reference materials purchased from an ISO 17025/17034 accredited supplier (Or equivalent) need only have their certificates of analysis checked and kept on file.

4.1.3 Qualitative Authentication for reference materials that are not from an approved 17025/17034 provider or do not come with a certificate of analysis.

4.1.3.1 Evaluate the mean retention time for the analyte using the analysis run data.

4.1.3.2 Compare volatile retention times reported for new reference material lot with retention time obtained from previous data.

4.1.3.3 The new lot can be accepted if the mean retention time for the new lot is ± 0.10 minutes.

4.1.3.4 For analytes of interest that have no previous data for comparison, those substances will be analyzed using structural analysis (GC/MSD, LC/MS, etc). Structural analysis needs to be performed by authorized personnel.

4.1.3.4.1 For unknown analytes of interest found in case samples, section 4.1.3.4 may be used to determine analyte identity in order to obtain a standard for comparison and identification through AM #1.

4.1.3.5 A standard will be considered structurally authenticated when the match (Q) is greater than 85 %, as compared to a library search and the analyst confirms that the spectra matches with no significant differences. If the spectra does not have a library match of 85% or greater the spectra may be authenticated by comparing it to a peer reviewed scientific journal, reference standard compendium or a library match that is less than a 85%. For these three options, two analysts trained to use the authentication instrumentation must initial the documentation signifying that it is an appropriate match.

4.2 Authentication of Matrix Controls

4.2.1 General

4.2.1.1 Refer to Blood Alcohol AM# 1 for GC-HS analysis requirements.

4.2.1.2 Matrix controls **must** be authenticated prior to being used in sample runs.

4.2.1.3 At least two analysts, each from a different laboratory, will run the new lot of control as if it were a case sample.

4.2.1.4 The data will be sent to the Discipline Leader for evaluation of the results.

4.2.1.5 The mean value obtained from analysis within the lab shall be the target value used for the new lot of matrix control.

4.3 Authentication Documentation

4.3.1 Reference Controls

Original authentication data will be maintained by the discipline leader. Documentation may be kept electronically.

4.3.2 Reference Material

A copy of all data used to authenticate the quantitative reference materials will be maintained by the alcohol discipline leader. Copies may be maintained in electronic format.

4.4 Safety Concerns

Biological samples must be processed and chemicals handled according to safety guidelines in the *Idaho State Police Forensic Services Health and Safety Manual.*

4.5 Quality Assurance

Refer to Blood Alcohol Analytical Method #1.

Analytical Method #3: Testing Guidelines

1.0 Background/References

1.1 In order to best utilize the resources available to Idaho State Police Forensic Services (ISPFS), whether analysis is performed and the degree of analysis pursued should be guided by all available information. It may not always be necessary and/or appropriate to proceed with sample analysis. For instance, when a valid breath test is obtained for a routine DUI, analysis of a blood sample for ethanol would not provide additional useful information. Another example is when analysis indicates a high blood alcohol value; additional testing for drugs other than ethanol requires extenuating circumstances.

2.0 Scope

2.1 This method addresses the factors to consider when determining the extent of analysis a volatiles case sample requires. The goal of these considerations is for the efficient utilization of resources in order to provide timely ethanol and other volatiles analysis results to user agencies.

3.0 Equipment/Reagents

3.1 None Associated directly with this Analytical Method

4.0 Procedure

4.1 Post-Blood Alcohol or Valid Breath Testing Analysis

4.1.1 When ISPFS laboratory analysis indicates that the ethanol concentration is 0.10/100cc or greater, further testing for additional drugs, in either blood or urine, should not be pursued unless justified by case related circumstances. This is in consideration that the legal limit for ethanol is 0.08 grams per 100 cc blood. Extenuating circumstances for additional toxicology testing may include the following: fatality crash sample from a living subject, sexual assault related cases, homicides or battery cases. In the case of crashes where the subject is the driver and is deceased and further toxicology testing is requested, testing will be performed on samples that have a blood alcohol content of less than 0.20 grams per 100 cc of blood.

4.1.2 If a breath test result is listed on the case submittal form or in pre-log, and no indication of a problem with the test is noted during the submission process, volatiles analysis will not be pursued. It is at the analyst's discretion to contact the agency to ascertain if extenuating circumstances exist when they are not indicated upon submission. If extenuating circumstances are indicated by the submitting agency, testing may be conducted on the sample.

4.1.3 Extenuating circumstances may include the following:

- Fatality or injury accident where additional volatiles use is suspected.
- Drug Recognition Exam (DRE) supports additional volatiles use. The DRE officer is reliant on a confirmation of their observations to maintain their certification.
- Volatiles related paraphernalia recovered from vehicle. Additional analysis could serve to support any additional charges.
- The breath testing instrument malfunctioned after the breath testing, preventing a valid performance verification from being obtained.
- In the case of crashes where the subject is the driver and is deceased and further toxicology testing is requested, testing will be performed on samples that have a blood alcohol content of less than 0.20 grams per 100 cc of blood.

4.1.4 The submitting officer or agency is responsible for providing justification for additional testing. Justification could take the form of a memo, e-mail or letter outlining the situation and a case report.

4.1.5 If the ethanol concentration is 0.10 or lower, future testing for other impairing drugs will not be pursued if the additional testing is not requested..

4.1.6 ISP will not analyze separator tubes, or tubes that appear to be non-homogenous by design.

4.1.7 In the event that IDAPA compliant tubes are submitted for analysis, other tubes submitted subsequent to the original submission will not be analyzed unless the original submission is deemed inadmissible for court purposes. Documentation from the court or prosecutors should be retained within the case record if such an analysis in performed.

4.1.8 When a combination kit containing multiple tubes and samples is submitted, it is as the discretion of the analyst on which sample to test. IDAPA compliance should be the main determining factor.

Analytical Method #4: POVA Intermediate Checks

1.0 Background/References

1.1 Upon receipt of a newly obtained pipette/dilutor and after maintenance performed on a POVA outside the laboratory or non-calibration maintenance performed in-house, the calibration must be verified to substantiate that the volume delivered is precise. This is accomplished by determining the repeatability of a mass of a volume of liquid of known density that has been delivered into a closed vessel. Maintenance is defined as any physical task performed on the instrument that would change the dynamics of the liquid pathway of the substances being displaced. In-house, this is a syringe change or a probe change.

1.2 References

- ASTM Method E-1154-89 (reapproved 2003), Standard Specification for Piston or Plunger Operated Volumetric Apparatus.
- Curtis, R.H., Performance Verification of Manual Action Pipets: Part I, Am. Clin. Lab. 12(7):8-9; 1994.
- Curtis, R.H., Performance Verification of Manual Action Pipets: Part II, Am. Clin. Lab. 12(9):16-17; 1994.
- Byer, B.J., How to Use and Check Pipetting Equipment, Scientific Newsletters, Inc., 1977.
- ISO 8655-6:2002, Piston-operated volumetric apparatus – Part 6: Gravimetric method for the determination of measurement error.

2.0 Scope

2.1 The reliability of the volume delivered by POVA is dependent upon verification of calibration. This method sets forth the requirements for both intermediate checks and calibration. The intermediate check is performed to maintain confidence in calibration. This manual weighing technique is an option to evaluate the performance of each POVA. The procedure is most applicable when larger volumes (≥1mL) are employed. This analytical method applies to air displacement pipettes as well as syringes attached to dilutors and dispensers. An approved external service provider performs actual POVA calibration.

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 Analytical Balance

- Capable of accurately weighing volumes of interest
- Note: Balance may be used if it has been checked using either the toxicology or controlled substances analytical method.

3.1.2 Weighing Vessel with Lid

- Nonporous material
- Assorted sizes to accommodate volume under consideration

3.2 Reagents

3.2.1 Deionized/distilled water

4.0 Procedure

4.1 Intermediate Check Procedure

4.1.1 General

4.1.1.1 Intermediate checks of the POVA's calibration are only required before initial use or after the instrument leaves the lab for maintenance/calibration.

4.1.1.1.1 Calibrations done by approved vendors within the laboratory do not require an intermediate check prior to use.

4.1.1.2 Each POVA should be tracked by its serial number and/or other unique identifier.

4.1.1.3 Intermediate checks of POVAs by an analyst or laboratory technician will be valid until the instrument is externally calibrated by an approved provider.

4.1.1.4 A POVA not in-use must be calibrated by an approved provider prior to use for an application that requires a calibrated POVA.

4.1.1.5 An intermediate check must be performed any time a POVA is serviced (Serviced is defined as non-calibration maintenance performed inside or any service performed outside the laboratory).

4.1.2 Initial set-up

4.1.2.1 The water used for the intermediate check process should be allowed to equilibrate at room temperature for at least two hours prior to the start of this procedure.

4.1.3 POVA Determinations

4.1.3.1 Use the designated POVA, to dispense appropriate volume (2.250 ml) of temperature-equilibrated water into the weighing vessel and cap/cover.

4.1.3.2 A minimum of ten individual repetitions should be recorded.

4.1.3.3 Calculate the Mean Delivered Weight, record on log sheet.

4.1.3.4 Calculate the Standard deviation of the samples and record on the spreadsheet

4.1.4 Evaluation of Accuracy and Precision

4.1.4.1 Acceptable performance of an intermediate check is accomplished if the three standard deviation value obtained from the replicate determinations is below 2% variation.

4.2 Calibration

4.2.1 All pipettes/dilutors crucial for the quality of quantitative analysis will be calibrated when analytical method quality control values and an intermediate check indicate unacceptable performance.

4.2.1.1 Auto-dilutors will be calibrated once per calendar year.

4.2.2 The calibration will be outsourced to an approved vendor/service provider.

4.2.2.1 Upon completion, the certificate will be checked and documented by an analyst with date and initials to indicate that it has passed within the specified acceptance criteria:

4.2.2.1.1 Precision: +/- 2%

4.2.2.1.2 Accuracy: +/- 5%

4.2.2.2 Certificates will be retained within the discipline, either electronically and centrally stored or in hard copy form within the laboratory.

4.2.2.3 If the instrument calibration parameters are not met, the instrument will be taken out of service, marked as out of service, and repaired or replaced.

Analytical Method #5: Criteria for Site Approval

1.0 Background/References

1.1 Section four of Idaho Code §18-8004 (Persons under the influence of alcohol, drugs or any other intoxicating substances) states the analysis of blood and urine for the purpose of determining the alcohol concentration shall be performed by a laboratory operated by the Idaho State Police or by a laboratory approved by the Idaho State Police under the provisions of approval and certification standards to be set by that department, or by any other method approved by the Idaho State Police. Idaho Administrative Code IDAPA 11, Title 03, Chapter 01 (11.03.01) outlines the requirements for a laboratory desiring to perform this analysis. IDAPA 11.03.01 states that the laboratory shall participate in approved proficiency testing and pass this proficiency testing according to standards set by the Idaho State Police Forensic Laboratory.

1.2 References

- IDAPA 11.03.01, Rules Governing Alcohol Testing.
- Idaho Statute §18-8004, Persons under the influence of alcohol, drugs or any other intoxicating substances.

2.0 Scope

2.1 As described above, a laboratory must take part in an Idaho State Police Forensic Services (ISP-FS) Laboratory recognized, proficiency testing program and be approved by the ISP-FS for start-up or to continue analysis of samples for alcohol content. This procedure describes the standards applied to this process.

3.0 Equipment/Reagents

3.1 None applicable for this Analytical Method

4.0 Procedure

4.1 PROCEDURE FOR TESTING SITE APPROVAL

4.1.1 Procedures Governing Analysis

IDAPA 11.03.01 requires each laboratory performing analysis for evidentiary purposes to prepare and maintain a written procedure governing its method of analysis, including quality control and proficiency testing guidelines. To verify conformity, a copy of the procedure must be provided to ISP-FS. Whenever protocol changes are adopted, a copy of the updated procedure must be forwarded to ISP-FS.

4.1.2 Proficiency Testing

4.1.2.1 ISP-FS approved providers include National Highway Transportation Safety Administration (NHTSA) and Collaborative Testing Services (CTS). Each test consists of at least four samples spiked with unknown concentration of ethyl alcohol.

4.1.2.1 Laboratories must participate in proficiency testing at least once a year. ISP-FS will only evaluate proficiency test results from approved providers.

4.1.2.2 Participating laboratories must obtain proficiency tests from approved providers and are responsible for all costs associated with obtaining and analyzing such tests.

4.1.2.3 Results from proficiency tests must be provided to the test provider and ISP-FS. Results not submitted to a test provider within the allowed time do not qualify as a proficiency test.

4.1.3 Evaluation of Proficiency Testing Results

4.1.3.1 An alcohol concentration range is determined from the target value as provided by the proficiency test provider. The acceptable range is the target value ± two standard deviations or 10%, whichever is greater. Reported values must fall within this range.

4.1.3.2 If a laboratory submits more than one alcohol value for a given sample the mean value of results will be evaluated.

4.1.4 Approval to Perform Legal Alcohol Testing

4.1.4.1 Upon satisfactory completion of an approved proficiency test, a letter and certificate of approval will be issued by ISP-FS to each participating laboratory.

4.1.4.2 Approval to perform legal blood alcohol determinations is continued until the results of the next proficiency test are reviewed and notification is sent to the respective laboratory by ISP-FS.

4.1.5 Disapproval to Perform Legal Alcohol Testing

4.1.5.1 Disapproval indicates that results are outside the tolerance range established from the accepted mean values.

4.1.5.2 When a laboratory fails to report values within the acceptable range, their approval to perform analysis on legal blood alcohol samples will be revoked.

4.1.5.3 A letter of disapproval will be issued by ISP-FS to the involved laboratory.

4.1.6 Reinstatement Following Disapproval

4.1.6.1 To be reinstated to perform alcohol analysis the laboratory must review their operation and satisfactorily complete a proficiency test approved by ISP-FS. For purposes of reinstatement, an approved test includes those described in section 4.1.2.

4.1.6.2 When a laboratory has successfully completed a second proficiency test, reviewed its operation, and the overall process has been evaluated by ISP-FS, the approval to perform legal alcohol determinations may be reinstated.

Analytical Method #6: Competency and Proficiency

1.0 Background/References

1.1 In accordance with the *Volatiles Analysis Training Plan,* a trainee will complete a competency test consisting of specimens which contain a representation of commonly encountered analytes. Thereafter, the analyst will complete an annual proficiency test. Breath Alcohol proficiency tests may be completed by reanalysis of a single instrument.

2.0 Scope

2.1 This method describes the criteria to be applied to the evaluation of results obtained for both competency and proficiency testing for ethanol and other volatiles analysis.

3.0 Equipment/Reagents

3.1 None Applicable to this Analytical Method Specifically

4.0 Procedure

4.1 Competency Tests

4.1.1 The competency test can be ordered through a reliable vendor or created internally.

4.1.2 The acceptable alcohol concentration range is determined from the target value provided by the manufacturer of the competency test or from internal testing.

4.1.3 Reported values must fall within ±10% of the target value reported by manufacturer.

4.1.4 If all volatiles are not detected and/or the quantitative ethanol value(s) reported does not fall within the allowable range, analysis procedures will be reviewed and additional training may be required as deemed appropriate by the Discipline Leader. The analyst will be required to perform an additional competency test.

4.2 Proficiency Tests

4.2.1 The blood alcohol proficiency test can be ordered through an approved vendor and/or Department of Transportation (DOT).

4.2.1.1 To comply with accreditation proficiency test requirements it is necessary for each laboratory to successfully complete one external test from an approved provider.

4.2.1.2 In order to comply with IDAPA 11.03.01 (approval to perform alcohol determinations for legal purposes), a laboratory must take part in an Idaho State Police Forensic Services (ISP-FS) recognized proficiency testing program.

4.2.1.3 A single proficiency test can be used to comply with both IDAPA and accreditation requirements as long as it is an approved test.

4.2.1.4 The appropriate tests to be ordered will be evaluated yearly by the Quality Assurance Manager with input from the Discipline Leader.

4.2.1.5 The acceptable alcohol concentration range is determined from the target value provided by the manufacturer of the competency test.

4.2.1.6 The target value will be based only on the compilation of results provided by accredited laboratories.

4.2.1.7 The acceptable range is the target value ± two standard deviations or 10%, whichever is greater.

4.2.1.8 Reported values must fall within this acceptable range.

4.2.1.9 If the value reported does not fall within the allowable range, analysis procedures will be reviewed and additional training may be required as deemed appropriate by the Discipline Leader. The analyst will be required to perform a competency test prior to resuming casework.

4.3 Competency Testing

4.3.1 The competency testing and proficiency testing only apply to the training/testing of volatiles analysis in fluids and does not apply to the breath alcohol program portion of the discipline. If an applicable breath testing proficiency/competency test is available through a commercial vendor or in agreement with AM #6 - 4.2.1, then that shall be the approved choice for proficiency testing and competency testing.

4.3.2 Until an approved vendor is available, the proficiency tests shall be created internally.

4.4 Maintaining Proficiency:

4.4.1 In order to maintain proficiency within the discipline, an analyst with less than five years of experience must complete a minimum of at least 4 BAC batch runs throughout the calendar year.

4.4.2 It is the responsibility of the analyst to keep track of their progress and proficiency, along with their supervisor. Updates should be provided at the discipline meetings for individuals that meet the criteria in 4.4.1.

Analytical Method #7: Uncertainty of Measurement

1.0 Background/References

1.1 Any measurement, no matter how carefully obtained, should not be considered as the true value for the measurement. Whenever any quantitative measurement is performed, the value obtained is only an approximation of the true value.[1] According to JCGM 200:2008, the International vocabulary of metrology – Basic and general concepts and associated terms (VIM),[3] measurement uncertainty is defined as "*A non-negative parameter associated with the result of a measurement/quantity value (*number *and measurement unit used together to express the magnitude of a quantity) that characterizes the dispersion of quantity values that could reasonably be attributed to the measurand (quantity intended to be measured).*" ISO/IEC 17025:2005 clause 5.4.6.2 requires that we make a reasonable estimation of uncertainty that is based on knowledge of the performance of the method and on the measurement scope and shall make use of for example, previous experience and validation data.[2] Clause 5.4.6.2, NOTE 1 goes on to state that the degree of rigor needed in an estimation of uncertainty of measurement depends on factors such as the existence of narrow limits on which decisions on conformity to a specification is based.[2] Paragraph 5.10.3.1 states that when applicable, the test report should include a statement on the estimated uncertainty of measurement.[2] For our purposes, it is applicable due to the uncertainty affecting the application of the test results which are compliant to a specification limit. In the analysis of forensic specimens, we do not know the true value for the specimen; hence this information is not the error associated with the analysis. Rather, it is a range of values likely to be encountered during the measurement process.[7] This information is crucial to the legal system because it impacts if and how an individual will be charged with an offense such as DUI.[4,5]

1.2 References:

- Huber, L., Validation and Qualification in Analytical Laboratories, pp. 146 - 150, Interpharm/CRC, 19910.
- International Organization of Standardization (ISO) / International Electrochemical Commission (IEC), *General requirements for the competence of testing and calibration laboratories*, 2005. (ISO/IEC 17025:2005)
- Joint Committee for Guides in Metrology (JCGM), International Vocabulary of Basic and General Terms in Metrology (VIM), 2008. (JCGM 200: 2008)
- Idaho Code §18-8004. Persons under the influence of alcohol, drugs or any other intoxicating substances.
- Idaho Code §18-8004C. Excessive Alcohol Concentration – Penalties.
- ISO/IEC 17025:2005: Section 5.4.6: Estimation of Uncertainty of Measurement Workshop, Presented by J.P. Bono and E.A. Mishalanie, AAFS 61st Annual Meeting, Denver, Colorado, 2009.
- Mason, F., Uncertain About Uncertainty, Quality Digest, Inside Metrology Column, 06-12-2008.

2.0 Scope

2.1 This analytical method will be applied to analytical methods which report quantitative results. This approach to uncertainty uses the standard deviation of controls and other known sources of uncertainty. A 99% confidence interval will be created by three standard deviations of data collected during the process. To properly represent the uncertainty, this data will be expressed as the Uncertainty of Measurement (UM) on the analysis report.

3.0 Equipment/Reagents

3.1 **Equipment:** Reference section 4.0 of this Analytical Method.

3.2 **Reagents:** Reference section 4.0 of this Analytical Method

3.3 **Quality Assurance Material:** Reference section 4.0 of this Analytical Method

4.0 Procedure

4.1 Reporting of Quantitative Ethanol Results

4.1.1 Analytical Methods

Analytical Method #1: Analysis of Volatiles by GC-HS

4.1.2 Determination of Confidence Interval

4.1.2.1 Control values obtained during the process are used to establish the UM based on the standard deviation of data as well as incorporating other known sources of uncertainty into the uncertainty budget. Aqueous controls, simulator solutions, or blood controls may be used to generate the data for the UM determination, as long as the standard is homogenous.

4.1.2.2 Three standard deviations will be calculated for a 99% confidence interval.

4.1.2.3 The mean value as determined by the above analytical method will be reported along with a ± UM.

4.2 Monitoring and Updating the Uncertainty of Measurement

4.2.1 Monitoring

4.2.1.1 The UM for the analysis process will be monitored per the Blood Alcohol AM# 1 through the use of certified reference materials. The reference materials shall be run with each batch of quantitative samples being analyzed and entered into a spreadsheet.

4.2.1.2 The results of the reference standards shall be reviewed annually. The review will consist of the Discipline Leader checking the results for each lab and issuing a summary of the results for the reference standard analysis.

Note: The memo shall consist of the following summaries at a minimum: Overall system standard deviation, overall system standard error, each regional laboratories overall standard deviation, and a quarterly breakdown of the standard deviation and standard error for each lab to identify trends.

4.2.2 Updating the UM for the system

4.2.2.1 Should a new GC-HS instrument be put into service within the laboratory, the measurement process for the affected laboratory shall be repeated using an available lot of control samples in the same prescribed manner as the original determination.

4.2.2.2 Should a new analyst be approved to perform volatile substance analysis, the measurement process will be performed by that analyst using an available lot of control samples in the same prescribed manner as the original determination.

4.2.2.3 Every four years, the process with be reproduced using a different lot of QC samples throughout the entire system. Each analyst that is approved and performing volatile substance analysis on blood and other fluids shall produce data used for the determination of the UM for the system. This process shall be substantially the same as the previous determinations and analyses.

4.2.2.4 When the UM is updated, reports that are in progress shall report the UM numbers in accordance with the version that is in effect during the ANALYSIS date found in the case notes, and not with the report issue date.