Section Four
Quality Assurance

5.14 Authentication of Reference Material and Matrix Controls: Volatiles

5.14.1 BACKGROUND
Refer to Analytical Method 4.1.

5.14.2 SCOPE
This method describes the Idaho State Police Forensic Services (ISP-FS) 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 for the presence of volatile compounds.

5.14.3 EQUIPMENT
5.14.3.1 Gas Chromatograph (GC) configured with Headspace Sampler (HS) and a Flame Ionization Detector (FID)
5.14.3.2 Gas Chromatograph (GC) configured with a Mass Selective Detector (MSD)
5.14.3.3 HS-GC-FID Columns
Restek Rtx®-BAC1 and Restek Rtx®-BAC2
5.14.3.4 GC-MSD Column
HP-5 MS or comparable
5.14.3.5 Headspace (HS) vials and Closures
5.14.3.6 Hand Crimper or Bench Top Crimper
5.14.3.7 Semi-Automatic Dilutor/Pipetter equipped with sample and reagent syringes capable of dispensing 250µL and 2000µL, respectively
5.14.3.8 Laboratory Oven
5.14.3.9 Gas tight syringe capable of dispensing 50µL

5.14.4 REAGENTS
5.14.4.1 Distilled/Deionized water (free from volatiles of interest)
5.14.4.2 Ammonium Sulfate (Certified ACS Grade)

5.14.5 REFERENCE MATERIAL
Refer to Analytical Methods 4.1 and 4.2.

5.14.6 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.*

5.14.7 QUALITY ASSURANCE

Refer to Analytical Method 4.1.

5.14.8 AUTHENTICATION OF REFERENCE MATERIALS

5.14.8.1 General

5.14.8.1.1 Refer to Analytical Method 4.1 for GC-HS analysis requirements.

5.14.8.1.2 Aqueous reference material used for quantitative purposes must be traceable to NIST standards.

5.14.8.1.3 All available *Certificates of Analysis* for reference material will be stored centrally.

5.14.8.1.4 New lots of aqueous ethanol, aqueous mixed volatiles, and volatile reagent reference material must be analyzed, in a minimum of duplicate, prior to official use.

5.14.8.2 Qualitative Authentication

5.14.8.2.1 New lots of aqueous ethanol, aqueous mixed volatiles and single component volatile reference material used to prepare multicomponent mixtures must be authenticated by GC-MSD prior to use.

5.14.5.2.2 The following volumes and temperatures are recommendations only. Other volumes and temperatures may be used in place of those listed.

5.14.8.2.3 **Neat Reference Material (Solvents)**
Place ≅10µL of neat reference material into a headspace vial and seal. Manually inject ≅50µL of room temperature vial headspace into GC with an airtight syringe.

5.14.8.2.4 **Aqueous Reference Material (Ampules)**
Place contents of ampule into a headspace vial, seal and heat at 60°C for approximately 5 minutes. Manually inject ≅50µL of vial headspace into GC with an airtight syringe.

5.14.8.2.5 GC parameters must be optimized for volatile being authenticated with consideration of boiling point. MSD parameters must be set such that appropriate ions are scanned for. The low scan range will most likely need to be lowered from default.

5.14.8.2.6 Library search and/or reference (article, book) must clearly indicate that the appropriate compound(s) are present. The authentication data must be centrally stored.

5.14.8.3 Quantitative Authentication

5.14.8.3.1 The *Certificate of Analysis* and quantitative data, compared to existing calibrators, will serve as the quantitative authentication of ethanol in a certified ethanol solution.

5.14.8.3.2 When a certified volatile reference solution contains components in addition to ethanol, only the ethanol concentration need be evaluated.

5.14.8.3.3 The new lot number of ethanol reference material can be accepted if the mean concentration obtained falls within 6% of the target value (assayed) listed on the *Certificate of Analysis*.

5.14.8.3.4 Evaluation of data must be such that compliance with concentration requirements is apparent.

5.14.8.3.5 If the volatile of interest is not listed in the following table, the volume added to a single component or multicomponent mixture must be optimized for the particular volatile and it must be verified that the compound does not coelute with other components. Documentation must be centrally stored.

Compound
Acetaldehyde
Acetone
Difluoroethane
Ethanol
Ethyl Acetate
Methanol
Isopropanol
Tetrafluoroethane
Toluene

5.14.9 AUTHENTICATION OF BLOOD MATRIX CONTROLS

5.14.9.1 General

5.14.9.1.1 The Toxicology Discipline Leader or designee will characterize a new lot of blood controls.

5.14.9.1.2 Each ISP-FS laboratory involved in alcohol/volatiles analysis will provide data for the authentication process.

5.14.9.1.3 Blood control *Package Inserts* will be stored centrally.

5.14.9.2 Blood Control Authentication Run Requirements

5.14.9.2.1 Each laboratory will provide a minimum of 20 samples (40 determinations).

5.14.9.2.2 The samples must be divided into at least two analysis runs.

5.14.9.2.3 At least three bottles of each control level must be sampled.

5.14.9.2.4 Ideally more than one analyst will generate data in each laboratory.

5.14.9.3 Blood Control Authentication Evaluation

5.14.9.3.1 The manufacturer's values will be acknowledged, however, the target value and ± range for blood control lot will be established through a four decimal place truncated mean of all provided determinations.

5.14.9.3.2 The new blood lot number can be accepted if the following requirements are met:

1. The mean relative retention time for the new control is ± 0.10 minutes of the RRT currently established for ethanol.

2. The mean concentration obtained falls within the range provided in the manufacturer's package insert.

5.14.9.3.3 A 10% and 5% range will be calculated from the mean value of the determinations and used to evaluate accuracy on subsequent analysis. The 5% range will serve as a warning limit.

5.14.9.3.4 For blood controls that contain other volatiles (e.g. acetone, methanol, isopropanol) in addition to ethanol, the qualitative determination of the components must be established through the comparison of relative retention times from the previous run. The values must agree within ± 0.10 minutes.

5.14.10 AUTHENTICATION DOCUMENTATION

5.14.10.1 Reference Material

Original authentication data and documentation of compliance with acceptance criteria will be maintained in the laboratory performing the authentication.

5.14.10.2 Matrix Controls

5.14.10.2.1 Original authentication data will be maintained in the laboratory performing the analysis.

5.14.10.2.2 A packet containing data from all matrix controls used for authentication and evaluation of the data will be maintained by the toxicology discipline leader.

5.14.11 REFERENCES AND RECOMMENDED READING

5.14.11.1 Stafford, D.T., *Chromatography. in:* Principles of Forensic Toxicology, edited by Barry Levine, pp. 91-98, 100-108, 114-118, AACC Press, 2006.

5.14.11.2 Levine, B. and Caplan, Y.H., *Alcohol. in:* Principles of Forensic Toxicology, edited by Barry Levine, pp. 169-184, AACC Press, 2006.

5.14.11.3 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.

5.14.11.4 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.

5.14.11.5 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.

Revision History

Section Five
Quality Assurance

5.14 Authentication of Reference Material and Matrix Controls: Volatiles

Revision #	Issue Date	Revisions
0	09-07-2009	Initial version. Separated from AM 4.1. Language and requirements updated.