Blood Alcohol Determination

Denver Police Department / Crime Lab

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Table of Contents

Document Control

Summary of Changes

Version #	Version Date	Nature of Change	Approver	Date Approved
1.0	08/26/2005	Initial Version	Donald A. Shriver	10/23/2005
1.1	0/12/2006	Revision	Donald A. Shriver	08/12/2006
1.2	12/07/2006	Revision – Assessment Finding	Donald A. Shriver	12/07/2006
1.3	04/04/2007	Revision – Regulation Changes	Donald A. Shriver	04/05/2007
1.4	05/08/2008	Revision – Regulation Changes; Record storage duration increased to 5 years.	Donald A. Shriver	05/08/2008
1.5	05/20/2009	Revision – Regulation Changes	Steve Sassetti	05/21/2009
1.6	10/01/2010	Revision – Calibration Change	Steve Sassetti	10/01/2010

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Document Review Plans

This document will be reviewed and updated, if necessary as defined below:

- As required to correct or enhance information content
- Following changes to the quality system standards
- Following any organizational changes or restructuring
- Following the State Department of Health Audit

Obtaining the most recent version of this document

The latest version of this document may be requested from the Chief of Police or Designee.

Document Distribution

This document is automatically distributed to all change approvers and upon request by the Director of the Crime Lab.

Security Classification

The security classification (Denver Police Department Confidential) and the handling of this document, comply with the Denver Police Department's Additional Policy Agreement. A record of this agreement can be obtained from the Human Resources Department of the Denver Police Department.

Management Review

Management reviews and approves this procedure and any required changes to it. Management will review this once every year.

Review Date	Comments	Next Review Date
10/12/2006	No changes-GSL	10/2007
10/24/2007	No changes - DAS	10/2008
11/172008	No changes – DAS	11/2009
11/10/2009	No changes – DAS	11/2010
11/30/2010	No changes – DAS	11/2011
11/28/2011	No changes – DAS	11/2012
11/20/2012	No changes – DAS	11/2013

Overview

This section summarizes the Blood Alcohol Determination procedure by defining the Blood Alcohol Determination procedure scope and objectives.

This procedure documents the steps taken to perform a Blood Alcohol Determination test for the identification of blood suspected of containing Ethanol.

This method is but one several possible methods for analysis of ethanol in blood. The general method described in this document has been approved by the Colorado State Health Department. This method was validated and accepted by the Colorado State Health Department and any literature that pertained to it would be kept by the Colorado State Health Department. Any other methodology approved by the Colorado State Health Department would be acceptable after appropriate validation studies

A sample of blood suspected of containing ethanol is placed in a vial containing an internal standard and Sodium Fluoride (NaF). The alcohol diffuses from the liquid phase into the vapor phase of the head space. A sample of the vapor in the head space is injected into a gas chromatograph after equilibrium had been established. The various volatiles are separated and ethanol is quantitated

Scope

The procedure includes the following capabilities:

- Materials needed
- Obtaining Evidence
- Calibration of GC
- Testing of sample
- Determining and reporting the results

This procedure begins with:

- Evidence obtained

This procedure ends with:

- Results of test being reported

Objectives

The objectives of the Blood Alcohol Determination are to:

- Handle Evidence properly
- Properly Calibrate the GC equipment
- Test and Identify the amount of Ethanol in a blood sample

Related Reference Documents

(*List any other documents which are referred to by this process or that are a useful reference for this procedure*)

Document Name	Document Purpose	Location
Manual for Analysis of Ethanol in Biological Liquids	Used to derive this testing method	*Dept. of Commerce 1977 DOT-TSC_NHTSA-76-4 (HS 802 208)*
Handbook of Chemistry and Physics	Used to derive this testing method	*63rd Edition 1982 -1983 CRC Press*
Committee Handbook	Used to derive this testing method	*National Safety Council Committee of Alcohol and other Drugs 1996*
Rules Pertaining to Testing for Alcohol and Other Drugs (Promulgated by the State Board of Health)	Used to derive this testing method	*5-CCR-1005-2 Last amended 01/21/09*
Blood Alcohol Methods 6890N	Instrument operating parameters	*Found on the 6890N in: C:\HPCHEM\1\METHODS\ALC6890N*
Blood Alcohol Methods 6890	Instrument operating parameters	*Found on the 6890 in: C:\HPCHEM\1\METHODS\ALC6890*

Roles and Responsibilities

This section describes responsibilities of those involved in delivering or supporting the Blood Alcohol Determination. The same individual might perform several roles. Responsibilities include, but are not limited to, those listed for each role.

Forensic Scientist

- Obtain sample
- Prepare sample
- Test sample according to this procedure
- Determine and report results of the test according to this procedure

Blood Alcohol Determination Procedure Steps

Prerequisite:	Evidence Collected
Inputs:	N/A
Outputs:	g of Ethyl Alcohol per 100mL of blood

Materials:

SPECIMEN REQUIRED:

A minimum of 1 ml. of whole human blood is preferred. Two samples should be collected using approved sterile 10 ml. vaccutainer tubes containing not less than 0.9% Sodium Fluoride per Colorado State Health Department recommendations. The tubes should be kept refrigerated at 2 to 8 degrees centigrade after collection.

STANDARDS:

The internal standard used is 0.1% n-propanol solution in distilled water. The calibration standards are NIST traceable standards prepared by Cerilliant. The values for calibration standards are 0.02 grams of ethanol, 0.08 grams of ethanol and 0.30 grams of ethanol per 100 mLs. The calibration will be verified by 0.02 grams of ethanol, 0.08 grams of ethanol and 0.30 grams of ethanol per 100 mLs NIST traceable standards prepared by Restek.

Equipment:

HP 6890 GC with HP7694 headspace sampler or 6890N GC with G1888 headspace sampler

Evidence Handling:

1. The Forensic Scientist will go to the Property Bureau located on the B1 level of the Police Headquarters Building.

2. The Forensic Scientist will ask the Property Technician for the Blood Alcohol Kits.

3. Blood Alcohol Kits are brought to the Crime Laboratory and each kit is inspected to insure the kit has been sealed with evidence tape. If the kit is not sealed this will be noted on the Toxicological request..

4. The toxicological request form will be removed from the kits one at a time and the kit number on the label of the kit will be noted on this form in the appropriate location. The information on the toxicological request form will be entered into the computer to obtain a laboratory number. The request form will be date stamped when received by the laboratory.

5. After the kit has been given a laboratory number one of the bar code labels will be placed onto the Toxicological request form and one placed onto the Blood Alcohol Kit. Two other labels are made to be placed onto the plastic bag the tubes will be placed in later.

6. A picture will be taken of the kit showing the laboratory number, etc. The top layer of the labels with the defendant's name etc. will be removed and these will be kept in the event the kit is destroyed before trial.

7. Blood kits will be opened one at a time and the tubes removed from the box and styrofoam insert. The tube to be tested will be put into a warm water bath to remove the DPD label. Once removed this label will be placed on the lower left hand corner of the toxicological request form. The tube will be labeled with the defendant's last name, lab number, analysts' initials and date. A 4 inch by 6 inch plastic bag will be sealed as to form two compartments and labeled with the lab bar code

label, defendant's last name, analysts' initials and date. The tube to be saved for the defense is placed into one of the compartments and after analysis the lab sample is placed into the other compartment and the entire bag is then sealed.

8. All variation from proper procedure concerning the blood kit will be noted on the toxicological request form, however all kits will be analyzed regardless of the condition of the sample. The weight of the evidentiary value of the sample will be left up to a court of law and not up to laboratory personnel.

9. The tube after being labeled etc. will be placed on the rocker to mix (at least 10 minutes).

10. The remaining kits will be treated the same way one at a time.

11. All objects (Kim wipes, pipet tips, gc vials, etc.) that come in contact with the blood and are disposable will be put into proper containers to be disposed. Disposal will consist of placing contaminated objects into proper containers and taking the container to the Property Bureau to be destroyed with other biological wastes by an authorized company under contract.

12. Samples will be returned to the Property Bureau and kept for at least one year from the date of offense under proper refrigeration, at less than 8˚C. Release of samples to outside laboratories, and the disposal of samples is the responsibility of the Property Bureau (procedures defined in the property bureau procedures manual).

Procedure:

Sample Preparation:

1. Allow all unknown human blood samples and standards and internal standards from the walk-in refrigerator to equilibrate to room temperature while gently mixing on a rocker (at least 10 minutes on rocker).

2. Prepare the needed forms (CHEMFORMBA2) (refer to evidence handling) and make item identification entries for the sequence program on the HP Chem station. Make a hard copy print out of the sample log table.

3. For each vial to be injected, place a minimum of 70mg of NaF into the 10 ml. autosampler vials. These vials will be used in routine order.

4. Using proper safety precautions (including lab coat, gloves, glasses, etc.) remove the stopper from the vaccutainer tube to be analyzed, or the vial containing the standard. Using a 2.5 ml. positive displacement repeater pipet, withdraw at least 0.5 ml. of blood or standard and dispense 200 µls into a 10ml. vial and then properly discard the pipet tip after each sample. Dispense 500 µls. of the internal standard using a 5 ml positive displacement repeater pipette into the same vial. Cap the vial with a Teflon septum and aluminum ring and crimp it closed. Vials should be numbered and then placed into proper sequence in the autosampler rack.

5. Verify that the HP 6890 or 6890N GC parameters are properly set and checked off on log. When a new instrument or method is to be brought online, documentation of validation will be kept on file. At least four samples of known concentrations (NIST traceable) will be used to validate any new instrument. When columns are replaced, at least four samples of known concentrations will be run to validate the new columns. Validation will include verification of accuracy, precision, the upper and lower limits of quantitation and column specificity (resolution of interferences).

Calibration of Equipment:

First the instrument is calibrated with two vials of the 0.02 ethanol standard (from Cerilliant), two vials of the 0.08 ethanol standard and two vials of the 0.30 ethanol standard (from Cerilliant). The calibration is checked by the analysis of the 0.02, 0.08 and 0.30 Restek standards. A blank will be run before the Restek standards and before each case. A 0.20 grams of ethanol per 100 mLs standard (Cerilliant) will be run at the conclusion of the sequence. See sample of blood alcohol sequence in Appendix A.

Results/Reporting:

The reported values will be an average of the results from all runs on a given sample (average of four on dual columns instruments and two on single column instruments). A result that is + or – 5% from the mean value of all results generated will be discarded. If the results from vial one to vial two on any given analysis set varies by more than 5%, an entire new analysis set will be prepared and run. Standards must be within 5% of the projected value (+/-0.002 for the 0.02 standards), or new vials will be prepared and run. Any value less than 0.02 grams of ethanol per 100 mLs. will be reported as 0 grams of ethanol per 100 mLs. If the results are greater than the highest standard in the run, 0.30, the results will be reported as greater than 0.30 g of ethanol per 100 mL of blood. After results are documented on the toxicological form it will be reviewed by another Forensic Scientist or the Forensic Science Section Supervisor and signed off on the Blood Alcohol Analysis Review Sheet (CHEMFORMBA1) showing the entire number of specimens and their results. A technical review will be completed by another Forensic Scientist on at least one batch of tests per month, and signed off on the Blood Alcohol Analysis Technical Review sheet (CHEMFORMBA3),

The analytical procedure used by the Denver Police Department Crime Laboratory has been evaluated (See Appendix B), and it has been determined that the 99.9% confidence interval is +/- 0.008 grams of ethyl alcohol per 100 milliliters of blood (see Blood Alcohol Determination SOP). This measurement of uncertainty will be stated on all reports and communicated to the Denver District Attorney's Office to be used in whatever manner they may deem appropriate.

A mixture of other volatiles will be run any time columns are replaced. This mixture will include acetone, methanol, ethanol, toluene, isopropyl alcohol, and n-propanol. If peaks other than ethanol and n-propanol are detected in a sample during a normal run then it can be identified by either the headspace GC or by GC-MS. It will not be quantified.

Quality Assurance:

A Q.A. log will be kept showing preparation of controls and standards, clerical errors and analytical errors or unusual results. An entry will be made in the Q.A. log any time testing is conducted. If no noteworthy event occurred, appropriate notation will be made to that effect. Additionally, an entry will be made in the log reflecting the measured value of the 0.20 standard.

The litigation packet is defined to contain copies of; the Toxicological Request and Report Form (DPD 134), the photo and peel off labels from the evidence collection kit box, the chromatograms from the sample tested, the chromatograms of all calibrators and standards run with the sample tested including the calibration table. All documents relating to blood alcohol testing are kept on file for five years.

Analyst competency, training and proficiency testing will be conducted in accordance with the Quality Assurance Manual.

Appendices

Appendix A

EXAMPLE OF BLOOD ALCOHOL SEQUENCE

VIAL#	SAMPLE NAME
1	0.02 (Cerilliant)
2	0.02 (Cerilliant)
3	0.30 (Cerilliant)
4	0.30 (Cerilliant)
5	0.08 (Cerilliant)
6	0.08 (Cerilliant)
7	blank
8	0.02 (Restek)
9	0.30 (Restek)
10	0.08 (Restek)
11	blank
12	sample #1
13	sample #1
14	blank
15	sample #2
16	sample #2
17	blank
18	sample #3
19	sample #3
20	blank
21	sample #4
22	sample #4
23	blank
24	sample #5
25	sample #5
26	blank
27	0.02 (Cerilliant)
28	0.08 (Cerilliant)
29	0.30 (Cerilliant)
30	0.02 (Restek)
31	0.08 (Restek)
32	0.30 (Restek)
33	0.20 (Cerilliant)
34	Blank

Appendix B

METHOD STATISTICAL VALIDATION.

Date:	Value of 0.200 Control (g/100mL)
3/1/2006	0.202
3/7/2006	0.204
3/13/2006	0.201
3/20/2006	0.202
3/29/2006	0.205
4/4/2006	0.203
4/11/2006	0.204
4/18/2006	0.202
4/19/2006	0.203
4/25/2006	0.209
5/3/2006	0.206
5/9/2006	0.21
5/16/2006	0.207
5/22/2006	0.206
5/30/2006	0.206
6/1/2006	0.209
6/5/2006	0.206
6/14/2006	0.205
6/15/2006	0.207
6/20/2006	0.208
6/27/2006	0.208
7/5/2006	0.208
7/6/2006	0.203
7/18/2006	0.209
7/21/2006	0.202
7/25/2006	0.208
8/1/2006	0.208
8/3/2006	0.208
8/4/2006	0.205
8/8/2006	0.208
8/16/2006	0.209
8/22/2006	0.204
	Standard Deviation: 0.002612
	Mean: 0.205781
	T Value: 2.04

Confidence Calculation at 99.9 percent (3σ)

3 x 0.002612 = 0.007836 or +/- 0.008