Date: December 4, 2014

To: Kris Cano, Lab Manager

From: P. Allan Kosecki, Forensic Scientist

Re: Validation Plan for Headspace Blood Alcohol Determination Using Agilent Gas Chromatograph

---

Purpose/Scope

The purpose of this validation study is to determine if the Agilent GC (US14173023) with autosampler (CN14160045) is appropriate for use in making blood alcohol determinations in the Toxicology Section using the methods developed in this study. This study will produce objective evidence to demonstrate that the requirements set forth in this plan were or were not met. The methods and instrument will be operated in the controlled environment of the toxicology examination room.

Materials/Instrumentation

Agilent GC model 7890B with dual FID
Agilent autosampler model 7697A
Agilent DB-ALC1 30-meter column
Agilent DB-ALC2 30-meter column
Aqueous ethanol controls
Whole blood ethanol controls
20 ml headspace vials with caps
Blank whole blood
Volatiles mixture
Hamilton Diluter/Dispenser
Hydrogen generator
Nitrogen generator
Air generator
0.015% w/v n-propanol

Sample Preparation

Samples will be prepared for analysis per the procedures documented in the Blood Alcohol Analysis Procedures Manual.

Analytical Method

The analysis will be a headspace analysis using n-propanol as an internal standard. Hydrogen will be used as the carrier gas. Headspace vials will be pressurized with nitrogen. The following settings will be used for the GC/autosampler:

GC oven:	40 °C
GC cycle	3.5 minutes

Front inlet	110 °C	
Pressure	10 psi	
Total flow	55.948 mL/min	
Split ratio	10:1	
FID settings:		300 °C
	H_2 flow	30 mL/min
	Air flow	400 mL/min
	Make up flow (N_2)	25 mL/min
Vial oven	60 °C	
Loop	60 °C	
Transfer line	90 °C	
Vial equil.	22 minutes	
Vial shaking	off	
Vial fill	15 psi	
Loop ramp	30 psi/min	

Performance Characteristics

The instrument/method will be evaluated using the following performance characteristics. Additional testing outside of the testing described in this plan may be done as part of evaluating the instrument's performance. Any additional testing will be detailed when describing the outcome of the validation testing. The retention time of a peak must be within 0.04 minutes of the retention time observed for ethanol in the calibration standards for a peak to be identified as ethanol.

- **Calibration model**

The applicability of a linear calibration model will be tested using data from five runs on five different days. Aqueous ethanol solutions will be used to assess the calibration model. The following calibrator concentrations will be used in each run: 0.02 g/dL, 0.10 g/dL, 0.15 g/dL, 0.20 g/dL, 0.30 g/dL, and 0.40 g/dL. The data points from the five runs will be plotted together to establish the calibration model. The origin will not be included as a calibration point.

The calibration model will be evaluated by looking at both the correlation coefficient (r) and the residuals. The r^2 values must be at least 0.995.

Outside of establishing the calibration model, the linearity of the detector response will be tested over a range of concentration from 0.01 g/dL to 0.50 g/dL. Three runs on three different days will be conducted using the following calibrator concentrations: 0.01 g/dL, 0.10 g/dL, 0.20 g/dL, 0.30 g/dL, 0.40 g/dL, and 0.50 g/dL. The linearity of detector response will be evaluated based on the correlation coefficient (r). The r^2 value must be at least 0.995.

- **Bias**

Bias will be evaluated using at least 3 separate samples per concentration per run in at least three separate runs. At least the following approximate ethanol concentrations will be used in the analysis of bias; 0.04 g/dL, 0.08 g/dL, 0.15 g/dL, 0.2 g/dL, and 0.4 g/dL. Both aqueous and whole blood reference materials will be used to assess bias. A bias of less than or equal to ± 5% is acceptable.

- **Precision**

Both within-run and between-run precision will be evaluated. Precision will be evaluated using at least 3 separate samples per concentration per run in at least three separate runs. At least, the following approximate ethanol concentrations will be used in the analysis of precision; 0.04 g/dL, 0.08 g/dL, 0.15 g/dL, 0.2 g/dL, and 0.4 g/dL. Both aqueous and whole blood reference materials will be used to assess precision. A percent coefficient of variation less than or equal to ± 10% is acceptable.

- **Carryover**

The potential for carryover will be evaluated by running a blank sample following a sample containing 0.50 g/dL ethanol. This test will be evaluated using three runs on three different days.

- **Interference studies**

Evaluating matrix interference

Human blood drawn into a vacutainer tube will be analyzed to evaluate the possibility of matrix interference. The blood sample will be analyzed without the presence of the internal standard to demonstrate the absence of interferences from the matrix.

Evaluating interference from other volatile organic compounds

The possible interference of the following volatiles will be studied: acetaldehyde, methanol, acetone, and isopropanol. The instrument must exhibit baseline separation of these volatiles from ethanol and n-propanol. In addition, the possible effect that a sample containing toluene might have an analysis of subsequent samples will be evaluated by analyzing control samples following samples containing toluene.

- **Limit of detection/Limit of quantitation**

The limit of detection will be determined by using the administratively-defined decision point of 0.01 g/dL. At least three samples of 0.01 g/dL ethanol solution per run will be examined on three different days. All detection and identification criteria must be met.

The limit of quantitation will be set as the lowest non-zero calibrator (i.e. 0.02 g/dL). At least three samples of 0.02 g/dL ethanol solution per run will be examined on three different days. All detection, identification, bias, and precision criteria must be met.

Documentation

The data generated during the validation study will be maintained and made available for review. The results of the validation study will be summarized and presented for review.