1.11 Measurements of Uncertainty

1.11 Measurements of Uncertainty

In an effort to comply with accreditation requirements and because scientific measurements, in general, are subject to variability, a budget estimating the uncertainty of measurement for alcohol and quantitative drug analysis is presented. An estimation of uncertainty shall be determined for all analytical procedures in the toxicology/blood alcohol unit in which a quantitative measurement is reported.

The uncertainty of measurement is defined as an estimate of the range of values within which the measured quantity is likely to lie. Defined another way, it is a quantitative method of expressing confidence in measurement.

1.11.1 Estimating the Uncertainty of Measurement

The uncertainty budget for this procedure shall include both random (Type A) uncertainties and systematic (Type B) uncertainties. Per ASCLD/LAB's Policy Measurement Uncertainty, section 5.3.1, the uncertainty will be reported to two significant figures. To be conservative, calculations used to estimate the uncertainty and the final combined uncertainty shall be rounded up. In order to combine the uncertainty, the uncertainty values shall be measured in the same units. In order to accomplish this, all uncertainties will be calculated as percentages.

1.11.1.1. Traceability

Traceability is established for all measurements through the use of log books in the laboratory. The use of all NIST traceable calibrators and controls are documented in these log books. By cross-referencing the date of analysis, one may determine the lot number, expiration date and date put into use of all NIST traceable certified reference solutions.

1.11.1.2. Random (Type A) Uncertainties

Type A uncertainty is defined as a method of evaluation of uncertainty by the statistical analysis of a series of observations. (GUM 2.3.2) Type A uncertainty is best determined by historical data of a large number of repeated measurements. The following historical data will be used to calculate the Type A uncertainty for associated methods:

- Blood Alcohol: Whole Blood Ethanol I and Whole Blood Ethanol II
- Toxicology: Low Positive and High Positive Controls

Control charts will be used to establish the historical standard deviation for each quantitative procedure. This standard deviation will be updated annually and will include all control samples run during that year. When monitoring two control samples for the same assay (ie: Whole Blood Ethanol I and II for alcohol analysis), the final combined uncertainty will be calculated using the larger of the two Type A uncertainties.

In the case of new procedures that lack historical control data, the control data from the validation of the new procedure may be used to establish the uncertainty measurement for the first year the procedure is in use.

When calculating the percent standard deviation in the case of multiple measurements on case specimens (as in blood alcohol analysis when the sample is run in duplicate), the standard deviation is divided by the square root of the number of measurements made on case specimens. Since each case is analyzed for blood alcohol in duplicate, the percent standard deviation used for the uncertainty measurement is divided by the square root of 2.

This calculation can be represented by the following formula: (mean) = __

p

Where: (mean) = standard deviation of mean

= historical standard deviation

p = number of measurements

1.11.1.3. Systematic (Type B) Uncertainties

Type B uncertainty is defined as a method of evaluation of uncertainty by means other than the statistical analysis of a series of observations. (GUM 2.3.3) Type B uncertainty has an equal chance of being any value within a particular range (-a to +a) and follows a rectangular (or uniform) distribution. Examples of Type B uncertainties would include:

- Volumetric flasks and pipettes
- Electronic pipettes
- Diluter/dispenser used for pipetting of samples in blood alcohol analysis
- Standard or calibrator reference material
- Graduated cylinders

The uncertainty associated with each of these variables is calculated by dividing the % value by the square root of 3, which results in the %

uncertainty.

This calculation can be represented by the following formula: $= \frac{a}{3}$

Where: = standard deviation

a = value of systematic uncertainty

Example: Value of error of dispeser/dilutor = 0.92%

$\frac{0.92}{3}$ = 0.53% systematic uncertainty

NOTE: Some sources of Type B uncertainty may be excluded based on the following NIST guideline: As a practical matter, the contributions of an input quantity to a measurement result is significant if a change in the value or uncertainty of the input quantity corresponds to a change in the significant figures of the stated values or uncertainty of the measurement result. Based on this NIST guideline, all Type B uncertainties whose percent uncertainty is calculated to be 0.449 % may be excluded from the combined uncertainty for the calculation of drug Toxicology uncertainties.

1.11.1.4. Combined Uncertainty

Type A and Type B Uncertainties are combined using the Root Sum Squares technique and the following formula:

Combined Uncertainty = $(A1^2 + B1^2 + B2^2 + B3^2 + B4^2 .....)$

Where A = Type A uncertainty and B = Type B uncertainty, both calculated as a percentage.

1.11.1.5. Determination of confidence

The combined uncertainty represents one standard deviation or a confidence level of about 68%, with a k value of 1. In order to determine the expanded uncertainty from the combined uncertainty, the combined uncertainty must by multiplied by the coverage factor (k) using this equation:

Uexpanded = Ucombined x k

The coverage factor at 95 % confidence is k = 2, and the coverage factor at 99.7 % confidence is k = 3.

If there is a lack of historical data, meaning fewer than 40 data points used in the calculation, the following Student's t table may be used to find the corrected coverage factor, based on the number of controls used to calculate the standard deviation. df = n - 1, where n = the number of controls analyzed.

dF	kcorr	dF	kcorr	dF	kcorr	dF	kcorr	dF	kcorr
1	127.3	8	3.83	15	3.28	22	3.11	29	3.03
2	14.09	9	3.69	16	3.25	23	3.10	30	3.03
3	7.45	10	3.58	17	3.22	24	3.09	40	2.97
4	5.59	11	3.49	18	3.19	25	3.07	50	2.93
5	4.77	12	3.42	19	3.17	26	3.06	60	2.91
6	4.31	13	3.37	20	3.15	27	3.05	80	2.88
7	4.02	14	3.32	21	3.13	28	3.04	100	2.87

The final calculated uncertainty measurement shall be calculated as a +/- %. If calculating a blood alcohol uncertainty, the two alcohol results shall be averaged and the uncertainty reported as a +/- percentage of the average.

1.11.2 Calculation of Uncertainty Budget for Blood Ethanol Concentration by Headspace GC

Details:

- Protocol **2.1 Determination of Ethanol (Ethyl Alcohol)**
- Measurand: Blood Ethanol
- All blood ethanol controls are logged in an Excel spreadsheet daily. This historical and statistical data is used to evaluate trends in the values of control samples. This data is also used in the calculation of Type A uncertainty.
- Equipment used: Headspace Gas Chromatographs

Type A:

1. Historical Values for Whole Blood Ethanol I Control

Type B:

1. NIST traceable Calibrators, purchased from Cerilliant

2. Dilutor/Dispenser

3. Acceptance Criteria for replicates as defined in the method

TABLE 1

Source of Uncertainty	Value (units)	Distribution	Divisor	Uncertainty
Type A- Historical data (n=480), SD = 0.001740037, target = 0.0773	2.3 %	Normal	1	2.3/2 = 1.6 %
Type B				
0.010 g/dL NIST traceable Calibrator (100.0 ± 0.4 ug/mL, to the 95% confidence level)	0.20 %	Rectangular	3	0.12 %
Dilutor/Dispensor (10.194 ± 0.064 uL)	0.63 %	Rectangular	3	0.36 %
Reproducability: (± 5% Acceptance criteria for replicate measurements)	5 %	Rectangular	3	2.9 %

Dilutor/Dispensor uncertainty is added to combined uncertainty twice- for the duplicate unknown samples pipetted in the alcohol procedure.

% SD = (Standard Deviation of population/target concentration of control) x 100

SD of Whole Blood Ethanol I = 0.001740037

Target concentration of WBE I = 0.0773 g/dL

(0.001740037/0.0773) x 100 = 2.3 %

2.3/2 = 1.6 %

Combined Uncertainty (type A and B) = $(1.6^2 + 0.12^2 + 0.36^2 + 0.28^2 + 2.9^2) = 3.3$ %

Combined Uncertainty x k (3) = 3.3 x 3 = 9.9 % = 9.9 %

To apply this uncertainty to casework, refer to the following example:

- A blood sample was run in duplicate and results of 0.153 g/dL ethanol and 0.159 g/dL ethanol were obtained.
- The average of these two results is 0.156 g/dL
- The uncertainty of the measurement is 0.156 g/dL ± 9.9%
- Calculated, the range of uncertainty in the measurement would be ± 0.015 (0.156 x 0.099 = 0.015)
- This results in a range of 0.141 g/dL – 0.171 g/dL for the uncertainty calculation.

To summarize, the measured uncertainty for blood alcohol analysis is ± 9.9 % to the 99.7 % confidence level.

1.11.3 Calculation of Uncertainty Budget for Drug Analysis by GC/MS

Details:

- Protocols **4.3.4 Quantitative Confirmation for Cannabinoids in Blood, 4.1.2 Quantitative Confirmation for Acidic, Neutral and Basic Drugs in Blood** and **4.2.1 Benzodiazapine Confirmation and Quantification in Blood**
- Measurand: Drug Toxicology Concentrations in blood
- All drug Toxicology controls are logged in an Excel spreadsheet. This historical and statistical data is used to evaluate trends in the values of control samples. This data is also used in the calculation of Type A uncertainty.
- Equipment used: Gas Chromatographs/Mass Spectrometers

Example Calculation for Blood THC Uncertainty:

Type A:

1. Historical Values for THC Low Positive Control

Type B:

1. Glass pipettes used to measure volume of blood sample

2. NIST traceable reference solutions, purchased from Cerilliant

Table 2

Source of Uncertainty	Value (units)	Distribution	Divisor	Uncertainty
Type A- Historical data (n=32), SD = 0.34, target = 3.0	11%	Normal	1	11%
Type B				
Volume of Sample				
2.0 ml glass transfer pipette 2.0 ml ± 0.03 ml	1.5%	Rectangular	3	0.87%
THC Cerilliant Standard Concentration				
1.000 mg/ml ± 0.043 (to the 95% confidence level)	2.2%	Rectangular	3	1.3%

% SD = (Standard Deviation of population/target concentration of control) x 100

SD of Low Pos THC = 0.34

Target concentration of Low Pos THC = 3.0 ng/ml

(0.34/3.0) x 100 = 11 %

Combined Uncertainty (type A and B) = $(11^2 + 0.87^2 + 1.3^2)$= 11%

Combined Uncertainty x k (3) = 11 x 3 = 33 % = 33%

To apply this uncertainty to casework, refer to the following example:

- A blood THC level of 15 ng/ml is measured.
- The uncertainty of the measurement is 15 ng/ml ± 33 %
- Calculated, the uncertainty of the measurement is ± 5 ng/ml

(15 x 0.33 = 4.95 so ± 5 ng/ml)

- This results in a range of 10- 20 ng/ml THC for the uncertainty declaration.

The following table (Table 3) summarizes the remaining uncertainty ranges for drugs quantified by the Michigan State Police Toxicology Unit.

Table 3

Drug Quantified	Combined Uncertainty (to 99.7% confidence level)
Alcohol	9.9 %
Blood THC	33 %
Blood THC-COOH	39 %
Alprazolam	42%
Amphetamine	51%
Benzoylecgonine	39%
Butalbital	24%
Carisoprodol	36%
Chlordiazepoxide	63%
Cocaine	36%
Codeine	51%
Diazepam	21%

Hydrocodone	39%
Meprobamate	39%
Methadone	90%
Methamphetamine	30%
Morphine	48%
Nordiazepam	33%
Oxycodone	39%
Phenobarbital	51%
Tramadol	33%
Zolpidem	36%
Blood GHB	72%
Urine GHB	78%
Clonazepam	27%
Lorazepam	39%
Oxazepam	21%
Temazepam	59%

1.11.4 Sources of Uncertainty (raw data and certificates)

2014 Type B Spreadsheets.pdf

Uncertainty spreadsheets

1.11.5 Resources

1. http://physics.nist.gov/Pubs/guidelines/
2. http://stattrek.com/Lesson3/Variability.aspx
3. ASCLD/LAB Policy on Measurement Uncertainty, AL-PD-3060 Ver 1.0, Effective Date: May 1, 2013
4. ASCLD/LAB Policy on Measurement Traceability, AL-PD-3057 Ver 1.0, Effective Date: May 1, 2013
5. http://physics.nist.gov/cuu/Uncertainty
6. GUM: Evaluation of measurement data- Guide to the expression of uncertainty in measurement, September 2008

2.1 Determination of Ethanol (Ethyl Alcohol)

2.1 Determination of Ethanol (Ethyl Alcohol) (Effective 1/2/2014)

2.1.1 General Description of Method

A procedure involving a gas chromatograph (GC) is utilized for the determination of ethanol. Two quantitative tests involving the addition of an internal standard to an aliquot of the liquid test sample is performed employing a head space injection technique.

2.1.2 Type and Size of Sample

Blood, urine, bile, vitreous humor, other liquid biological sample or alcoholic solution may be used for analysis. A 50 microliter aliquot of sample is used for the quantitative tests. When two blood samples are received on an individual, perform both GC analyses on the one sample closer to the time of the incident. For OWI cases, when two urine samples are received that were voided more than 5 minutes apart, perform both GC analyses on the second or latter urine sample. For all other cases, perform GC analyses on the first or earlier urine sample.

2.1.3 Equipment and Reagents

2.1.3.1

Thermo Scientific Trace gas chromatographs (Trace GC), each fitted with a flame ionization detector, a CTC Analytics CombiPal or Tri-Plus RSH autosampler, and PC based data system.

2.1.3.2 Required standards:

2.1.3.2.1 Internal Standard Solutions:

- 1 - Propanol, 0.02 gm/dl in deionized water
  (To be used for Rtx-BAC 1 or Rtx-BAC Plus 1 Columns)
- t - Butanol, 0.0078 gm/dl in deionized water
  (To be used for Rtx-BAC 2 or Rtx-BAC Plus 2 Columns)

2.1.3.2.2 Aqueous calibrators purchased from Cerilliant (multicomponent calibrators contain ethanol, methanol, isopropanol, and acetone):

- 0.010 g/dL multicomponent calibrator
- 0.050 g/dL multicomponent calibrator
- 0.100 g/dL multicomponent calibrator
- 0.200 g/dL ethanol calibrator
- 0.400 g/dL multicomponent calibrator
- 0.500 g/dL ethanol calibrator

2.1.3.2.3 Aqueous ethanol controls purchased from Cerilliant:

- 0.020 g/dL ethanol
- 0.080 g/dL ethanol
- 0.150 g/dL ethanol
- 0.300 g/dL ethanol
- 0.400 g/dL ethanol

2.1.3.2.4 Additional Controls

- Aqueous Volatile Mix Control (0.100 g/dL each of methanol, ethanol, isopropanol and acetone)
- Aqueous Low Volatile Mix Control (0.010 g/dL each of methanol, ethanol, isopropanol and acetone)
- Human Whole Blood Ethanol Controls
- Negative Control (deionized water)

2.1.3.3

Disposable gloves and eye protection from biohazards.

2.1.3.4

Biological safety cabinet.

2.1.3.5

Automatic pipettor-dilutors.

2.1.3.6

20 mL size autosampler vials with butyl rubber septa and metal caps.

2.1.3.7

Crimper for sealing metal caps.

2.1.3.8

Labquake Shaker sample mixing apparatus.

2.1.3.9

Assorted support equipment as needed; beakers, disposable and volumetric pipettes, flasks, tissue grinders, tissue wipes, protective gloves, etc.

2.1.4 Types of Columns

- Rtx-BAC 1 or Rtx-BAC Plus 1 (Restek; 30 meter X 0.53 mm)
  Recommended for use with 1-Propanol internal standard solution.
- Rtx-BAC 2 or Rtx-BAC Plus 2 (Restek; 30 meter X 0.53 mm)
  Recommended for use with t-Butanol internal standard solution.

Pertinent information about the columns and GC temperatures will be found on the chromatograms.
The oven, injector and detector temperatures used in unknown testing shall be the same as that used for its calibration.
NOTE: The actual column temperature may vary from column to column as needed to maintain acceptable analysis time and retention time consistency for the analytes.

2.1.5 Method

At the beginning of each day, inspect all equipment for proper function and cleanliness, and repair or replace parts when necessary. Verify that sufficient reagents and compressed gases are available for the day's work.

Quantitative testing is performed on two Trace GCs, each with a different internal standard.

These instruments are calibrated each day a batch is run, using the concentrations of ethanol, methanol, isopropanol and acetone from the list above to generate calibration curves. When possible, the Cerilliant (NIST traceable) Ethanol Controls, Human Whole Blood Ethanol Controls, the Aqueous Volatile Mix and the Aqueous Low Volatile Mix shall be run following calibration within the batch run as "unknowns" to verify the calibration curve. These control samples will be used to verify and insure the proper function and accuracy of the instrument, the method, and the calibration curve.

New lots of Human Whole Blood Ethanol Controls should be analyzed prior to being put in use.

The calibration models and peak detection algorithims for the Trace GC Ultra are as follows:

Analyte	Curve Type	Weighting	Origin	Peak Detection Algorithm
Ethanol	Linear	Equal	Ignore	Avalon
Methanol	Linear	Equal	Ignore	Avalon
Acetone	Linear	Equal	Ignore	Avalon
Isopropanol	Linear	Equal	Ignore	Avalon

The calibration models and peak detection algorithms for the Trace 1310 GC are as follows:

Analyte	Curve Type	Weighting	Origin	Peak Detection Algorithm

Ethanol	Quadratic	Inverse (1/x)	Ignore	Avalon
Methanol	Linear	Inverse (1/x)	Ignore	Avalon
Acetone	Linear	Inverse (1/x)	Ignore	Avalon
Isopropanol	Linear	Inverse (1/x)	Ignore	Avalon

Calibration models shall not be changed in the processing method or Quan Browser unless validated.

A homogeneous blood sample is assured by gently rocking the specimen on the Labquake Shaker for at least 5 minutes. If the specimen is clotted, homogenizing glassware can be used to obtain a liquid sample. All sample handling will be performed in the biological safety cabinet using the universal biohazard handling techniques. Only one tube of the submitted sample(s), normally, is tested on each GC instrument. The other, if present, is left in its unopened state.

Each control and unknown biological specimen is processed in the biological safety cabinet by using the two techniques that follow. At the end of each day's testing, all potentially contaminated equipment will be decontaminated with approved solutions provided for that purpose.

2.1.5.1 Biological Specimens

- A 50 ul aliquot of the sample is aspirated by the automatic pipettor-dilutor, dispensed and rinsed with 800 ul of the 1-propanol alcohol internal standard solution into a 20 ml size autosampler vial with butyl rubber septa and metal crimp cap. The vial is placed in its designated position in the autosampler according to the itemized sample list. A headspace sample of this mixture is injected into the appropriate GC.
- A 50 ul aliquot of the sample is aspirated by the automatic pipettor-diluter, dispensed and rinsed with 800 ul of the t-butanol alcohol internal standard solution into a 20 ml size autosampler vial with butyl rubber septa and metal crimp cap. The vial is placed in its designated position in the autosampler according to the itemized sample list. A headspace sample of this mixture is injected into the appropriate GC.

2.1.5.2 Alcoholic Beverages

Samples identified as suspected alcoholic beverages should be diluted in the manner described below before analysis.

2.1.5.2.1

For beer or beverages like beer, dilute the sample at least in the ratio 1:10

2.5.1.2.2

For spirits or beverages like spirits, dilute the sample at least in the ratio 1:100
Proceed with the analysis as shown for biological specimens above. All beverage samples shall have a negative control run following them to rule out any possible carryover.

2.1.6 Interpretation of Data

Chromatograms from each of the instruments are collated and reviewed to insure proper instrument function. The identity of the detected analyte(s) from the chromatogram is established by the agreement of the retention time with previously run standards. The analyst shall bring all chromatographic irregularities to the attention of the supervisor. The data may be re-processed manually on a case-by-case basis. The quantitative results of the analyte as indicated on the chromatogram from each instrument are evaluated.

2.1.6.1 Controls

Correlation of determination (r2). The r2 value for the ethanol linear regression curve must be 0.9990.

Acceptable tolerance for positive ethanol controls is ±5% of the target concentration or ±0.005 g/dL, whichever is greater. Acceptable tolerance for methanol, acetone and isopropanol positive controls is ±10% of the target concentration or ±0.005 g/dL, whichever is greater.

- The target concentration of an external control is defined as the manufacturer's intended value for that control. The target concentration can be listed as, but is not limited to, "Target Value", "Mean" or "Concentration".
- The target concentration of an internally prepared control is defined as the level at which that control has been prepared.

If one control does not meet acceptance criteria on one instrument, no action is necessary. If more than one control does not meet acceptance criteria on one instrument, the analyst will notify the supervisor. The supervisor will determine whether a portion of the run or the entire run will be re-analyzed.

2.1.6.2 Casework

The validated limit of quantitation for ethanol is 0.010 g/dL. The validated limit of detected for ethanol is 0.005 g/dL. The linear reportable range for quantitative ethanol results is 0.010 g/dL to 0.500 g/dL. (See section 2.1.8 for reporting guidelines)

2.1.6.2.1 Ethanol in Biological Specimens

2.1.6.2.1.1 Ethanol concentration is 0.010 g/dL and 0.500 g/dL on both columns:

- The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
- See link to the spreadsheet below for acceptance criteria:

Alcohol Acceptance Criteria

2.1.6.2.1.2 Ethanol concentration is <0.010 g/dL and 0.005 g/dL on one or both columns (where both results are 0.005 g/dL):

- The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
- If the average of the two results is 0.010 g/dL, with one of the two results at a concentration <0.010 g/dL (Limit of Quantitation), the case shall be reported as: **Ethanol present at a concentration 0.010 grams per 100 milliliters of blood** (or applicable matrix and result concentration).
- If the average of the two results is <0.010 g/dL and 0.005 g/dL, the case shall be reported as: **Ethanol present at a concentration <0.010 grams per 100 milliliters of blood** (or applicable matrix).

2.1.6.2.1.3 Ethanol concentration is <0.005 g/dL on one or both colums:

- Ethanol is reported as 0.000 grams ethanol per 100 milliliters of blood.

2.1.6.2.1.4 Ethanol concentration is >0.500 g/dL on one or both columns:

- The sample should be repeated on both columns using a 1:2 (or other appropriate) dilution.
- The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
- Apply the appropriate dilution factor to the results obtained on each chromatogram to calculate the concentration of the samples.

2.1.6.2.2 Methanol, Isopropanol and Acetone in Biological Specimens

- The linear reportable range for methanol, isopropanol and acetone is 10 mg/dL to 400 mg/dL (0.010 g/dL to 0.400 g/dL).
- The analyte(s) must be present on both columns in the following concentration(s) for a positive quantitative result to be reported:
    - Methanol 10 mg/dL (0.010 g/dL)
    - Isopropanol 10 mg/dL (0.010 g/dL)
    - Acetone 10 mg/dL (0.010 g/dL)
- The results of the two tests are averaged. The difference between the average and either of the two results should not exceed ±10% or ±5 mg/dL (0.005 g/dL), whichever is greater.
- Results of <10 mg/dL (0.010 g/dL) on one or both columns are not reported.
- If the methanol, acetone and/or isopropanol concentration is >0.400 g/dL on one or both columns:
    - The sample should be repeated on both columns using a 1:2 (or other appropriate) dilution.
    - The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
    - Apply the appropriate dilution factor to the results obtained on each chromatogram to calculate the concentration of the samples.

2.1.6.2.3 Alcoholic Beverages

- Results from the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
- Apply the appropriate dilution factor to results obtained on each chromatogram.
- Divide the calculated result by 0.79 (specific gravity of ethanol) to convert the result into percent volume/volume.
- The average of the two results is used to determine if the sample has a concentration of greater than or equal to 0.5% by volume.

2.1.6.3 Records

Calibrator and control data shall be maintained by the analyst.

An Alcohol Batch Worksheet should be completed and maintained with the calibrator and control data.

Each case file shall contain all scientifically valid chromatograms specifically associated with that case.

2.1.7 Alternate Procedure When an Instrument is Non-Functional

Perform the testing in duplicate on a functioning gas chromatograph.

2.1.8 Reporting the Results of Alcohol Testing

In compliance with sections 625a, et seq., of Act No. 300, the following guidelines are to be used for reporting alcohol results from the Toxicology Unit.

2.1.8.1 Blood Alcohol

2.1.8.1.1 Ethanol Results Reported 0.010 g/dL (where both results are 0.010 g/dL)

- The results from both chromatograms are entered to 3 decimal places into the Alcohol Worksheet in Forensic Advantage LIMS.
- If a result has been calculated due to a dilution, the calculated results are entered to 3 decimal places in the Alcohol Worksheet in Forensic Advantage LIMS.
- The results are displayed to 3 decimal places and shall be reported with the unit of measurement **"grams alcohol per 100 milliliters blood".**
- The calculated uncertainty statement appears below the results and appears on the final report in the format **"The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 100 milliliters blood at the 99.7% level of confidence."**

2.1.8.1.2 Ethanol Results Reported where at least one result is <0.010 g/dL and 0.005 g/dL (where both results are 0.005 g/dL)

- The following statement is typed into the Narrative field: **Ethanol present at a concentration <** (or , as applicable) **0.010 (or applicable concentration) grams alcohol per 100 milliliters blood** (or applicable matrix).
- No uncertainty statement is required.

2.1.8.1.3 Ethanol Results Reported <0.005 g/dL

- Both results are entered into the Blood Alcohol Worksheet as 0 (zero) g/dL.
- The results are displayed to 3 decimal places and reported with the unit of measurement **"grams alcohol per 100 milliliters blood".**
- The uncertainty statement will be removed from the Result Worksheet and should not appear on the final report.

2.1.8.2 Urine Alcohol

2.1.8.2.1 Driving related cases (reported in units: g/67 mL)

2.1.8.2.1.1 Ethanol Results 0.010 g/dL (where both results are 0.010 g/dL)

- The individual positive quantitative urine alcohol results from each chromatogram will be multiplied by a factor of 0.67.
- The calculated results will then be entered to 3 decimal places into the in the Alcohol Worksheet in Forensic Advantage LIMS.
- The results are displayed to 3 decimal places and shall be reported with the unit of measurement **"grams alcohol per 67 milliliters urine".**
- The calculated uncertainty statement appears below the results and appears on the final report in the format **"The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 67 milliliters urine at the 99.7% level of confidence."**
- No further conversion of urine alcohol into equivalent whole blood alcohol concentration is necessary.
- Reference to the time the urine was collected will not be included in the final report.

2.1.8.2.1.2 Ethanol Results <0.010 g/dL and 0.005 g/dL on one or both columns (where both results are 0.005 g/dL)

- The individual positive quantitative urine alcohol results from each chromatogram do not need to be multiplied by 0.67, as no quantitative results will be reported.
- The following statement is typed into the Narrative field: **Ethanol present at a concentration <** (or , as applicable) **0.010** (or applicable concentration) **grams alcohol per 100 milliliters urine.**
- No uncertainty statement is required.

2.1.8.2.1.3 Ethanol Results <0.005 g/dL

- Both results are entered into the Alcohol Worksheet in Forensic Advantage as 0 (zero).
- The results are displayed to 3 decimal places and reported with the unit of measurement **"grams alcohol per 67 milliliters urine".**
- The uncertainty statement is to be removed from the Result Worksheet and should not appear on the final report.

EXAMPLE:

A urine alcohol concentration of 0.249 obtained from one chromatogram is calculated as follows: (0.249 X 0.67 = 0.167). The result of 0.167 is then entered in the Alcohol Worksheet. The same calculation is performed for the second result of 0.255 (0.255 x 0.67 = 0.171) and the calculated result of 0.171 is entered in the Alcohol Worksheet. The urine alcohol result will now be reported as: "0.169 grams alcohol per 67 milliliters urine"

2.1.8.2.2 Non-driving related cases including sexual assault and decedents

2.1.8.2.2.1 Ethanol Results Reported 0.010 g/dL

- The results from both chromatograms are entered to 3 decimal places into the Alcohol Worksheet in Forensic Advantage LIMS.
- The results are displayed to 3 decimal places and shall be reported with the unit of measurement **"grams alcohol per 100 milliliters urine".**
- The calculated uncertainty statement appears below the results and appears on the final report in the format "**The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 100 milliliters urine at the 99.7% level of confidence.**"

2.1.8.2.2.2 Ethanol Results Reported where at least one result is <0.010 g/dL and 0.005 g/dL (where both results are 0.005 g/dL)

- The following statement is typed into the Narrative field: **Ethanol present at a concentration <** (or , as applicable) **0.010** (or applicable concentration) **grams alcohol per 100 milliliters urine.**
- No uncertainty statement is required.

2.1.8.2.2.3 Ethanol Results Reported <0.005 g/dL

- Both results are entered into the Blood Alcohol Worksheet as 0 (zero) g/dL.
- The results are displayed to 3 decimal places and reported with the unit of measurement **"grams alcohol per 100 milliliters urine".**
- The uncertainty statement is to be removed from the Result Worksheet and should not appear on the final report.

2.1.8.3 Serum or Plasma Alcohol

2.1.8.3.1 Ethanol Results Reported 0.010 g/dL

- The results from both chromatograms are entered to 3 decimal places into the Alcohol Worksheet in Forensic Advantage LIMS.
- Additional calculations and a reporting step must be performed to convert the serum or plasma result to an equivalent whole blood alcohol concentration and calculate the uncertainty:
    - The average result from the Alcohol Worksheet is divided by 1.16 to obtain the whole blood alcohol equivalent.
    - The re-calculated uncertainty is obtained by multiplying the whole blood equivalent result by the current expanded uncertainty.
    - Results are entered into the Narrative section of the Alcohol Worksheet, see below example for format.

EXAMPLE:

Result from chromatogram #1: 0.249 g/dL serum

Result from chromatogram #2: 0.255 g/dL serum

Average of results: 0.252 g/dL serum

The two results agree within ±5% of the average, both results are entered into the Alcohol Worksheet.

The average from above is divided by 1.16 to obtain the whole blood alcohol concentration equivalent:

0.252 / 1.16 = *0.217*

The uncertainty must be re-calculated by multiplying the whole blood concentration equivalent by the current expanded uncertainty:

Current expanded uncertainty: 9.6% = 0.096

The uncertainty of the whole blood alcohol equivalent is calculated:

0.217 x *0.096* = *0.021*

In the narrative field of the Alcohol Worksheet result and uncertainty statements are entered in the following format:

- The concentration of this serum (or plasma) alcohol is equivalent to 0.217 grams alcohol per 100 milliliters whole blood.
- The calculated uncertainty of the alcohol measurement is estimated to be equivalent to ±0.021 grams alcohol per 100 milliliters whole blood to the 99.7% level of confidence.

- The results are displayed to 3 decimal places and shall be reported with the unit of measurement **"grams alcohol per 100 milliliters serum".**
- The calculated uncertainty statement appears below the results and appears on the final report in the in the format "**The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 100 milliliters serum at the 99.7% level of confidence.**"
- The statements entered into the narrative field display below the uncertainty statement.

2.1.8.3.2 Ethanol Results Reported where at least one result is <0.010 g/dL and 0.005 g/dL (where both results are 0.005 g/dL)

- The following statements are typed into the Narrative field: **Ethanol present at a concentration <** (or , as applicable) **0.010** (or

applicable concentration) **grams alcohol per 100 milliliters serum. The concentration of this serum (or plasma) alcohol is equivalent to an alcohol concentration <** (or , as applicable) **0.010** (or applicable concentration) **grams alcohol per 100 milliliters blood.**

- No uncertainty statement is required.

2.1.8.3.3 Ethanol Results Reported <0.005 g/dL

- Both results are entered into the Blood Alcohol Worksheet as 0 (zero) g/dL.
- The following statement is entered into the Narrative field:
  - The concentration of this serum (or plasma) alcohol is equivalent to 0.000 grams alcohol per 100 milliliters whole blood
- The results are displayed to 3 decimal places and reported with the unit of measurement **"grams alcohol per 100 milliliters serum".**
- The uncertainty statement will be removed from the Result Worksheet and should not appear on the final report.

2.1.8.4 Methanol, Acetone and Isopropanol in Biological Specimens

- Both results are entered into the Blood Alcohol Worksheet as whole numbers (no decimal places).
- The results are displayed to three decimal places, but must be edited to remove decimal and digits to the right of decimal.
- The result(s) shall be reported as a whole number(s) (no decimal places) with the units **"milligrams (analyte) per 100 milliliters (matrix)."**
- The uncertainty statement will be removed from the Result Worksheet and should not appear on the final report.

2.1.8.5 Alcoholic Beverages

- The average of the two results calculated in 2.1.6.2.3 is entered into the Alcohol Worksheet in the Beverage Ethanol Result field.
- If the result of analysis is 0.5% v/v the following statement is reported:

  "Analysis of the sample showed the presence of greater than 0.5% by volume ethyl alcohol and its composition is consistent with an alcoholic beverage".

- If the result of analysis is <0.5% v/v the following statement is reported:

  "Analysis of the sample showed the presence of less than 0.5% by volume ethyl alcohol and its composition is not consistent with an alcoholic beverage".

- Uncertainty will not be reported with alcoholic beverage results.

2.1.9 Bibliography

There are several good review articles on alcohol testing that show the general scientific acceptability of the method detailed here.

Jones AW: Measuring Alcohol in Blood and Breath for Forensic Purposes - A Historical Review; Forensic Sci Rev 8: 13; 1996

Tagliaro F, Lubli G, Ghielmi S, Franchi D, Marigo M: Chromatographic Methods for Blood Alcohol Determination; J Chromatography 580:161; 1992

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