1.11 Measurement 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 Measurement Uncertainty

The uncertainty budget for this procedure shall include both Type A and Type B uncertainty components. 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

Measurement traceability is defined as the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

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. Type A Evaluation

Type A evaluation is defined as a method of evaluation of uncertainty components 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, Medium and High Positive Controls

Multiple trained, competent analysts within the Toxicology Unit perform casework, on a number of different instruments. Because of this, there are uncertainty components that are inherently able to be evaluated when statistically analyzing the repeated measurements of the controls analyzed with that casework. The following uncertainty components are considered to have been evaluated when analyzing historical control values:

- Multiple analysts
- Training of analysts
- Experience of analysts
- Matrix of sample
- Stability of matrix
- Temperature of samples
- Variability of temperature in preparation room
- Variability of humidity in preparation room
- Variation in the use of the pipette diluter
- Variation in use of pipettes
- Variation in autosampler vial crimping technique
- Variation in SPE/LLE extraction technique
- Variation in turbovap settings
- Instrument precision
- Instrument parameter settings
- Stability of samples from preparation through analysis
- Calibration model

Control charts will be used to establish the historical standard deviation and mean 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: $\sigma\,(\text{mean}) = \frac{\sigma}{\sqrt{p}}$

Where: σ (mean) = standard deviation of mean

σ = historical standard deviation

p = number of measurements

1.11.1.3. Type B Evaluation

Type B evaluation is defined as a method of evaluation of uncertainty components 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
- 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: $\sigma = \frac{a}{\sqrt{3}}$

Where: σ = standard deviation

a = value of systematic uncertainty

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

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

Calibrators
While calibrators are considered a type B uncertainty, the uncertainty associated with them assumes a normal distribution.

Example:

Uncertainty of calibrator from COA = 100.0 ± 0.4 µg/mL with a K=2

Relative uncertainty = 0.00004 g/dL / 0.010 g/dL * 100 = 0.40%

Relative standard uncertainty = 0.40% / 2 = 0.20%

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:

$$\text{Combined Uncertainty} = \sqrt{(A1^2 + B1^2 + B2^2 + B3^2 + B4^2 \ldots..)}$$

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:

$$U_{expanded} = U_{combined} \times k$$

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	3.1	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

EXAMPLE CALCULATION:

Source of Uncertainty (Type A)	Value	Distribution	Divisor	Uncertainty
Alcohol Historical Data (n=488)	2.73%	Normal	√2	1.93%
mean of the data set 0.201, std dev 0.005490				
RSD = std dev/mean of the data set = 0.027313432				
%RSD = RSD*100 = 2.73%				

Source of Uncertainty (Type B)	Value	Distribution	Divisor	Uncertainty
Certified Reference Material	0.50%	Normal	2	0.25%
0.01 Calibrator (100.0 ug/mL ± 0.50 ug/mL)				
Dilutor/Dispensor Calibration	0.46%	Rectangular	√3	0.27%
Target 50 µL, mean 49.77 µL				
Bias = (49.77-50)/50 = 0.0046				
%Bias = 0.0046*100 = 0.46%				
Reproducability of Replicates	5.00 %	Rectangular	√3	2.9 %
± 5% of replicate measurements				

Combined Uncertainty = $\sqrt{(1.93^2 + 0.25^2 + 0.27^2 + 2.9^2)}$= 3.5 %

n = 488

3.5% x k3 = 10.5%

Ethyl Alcohol Uncertainty to 99.7% confidence level = 10.5%

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 ± 10.5%

Calculated, the range of uncertainty in the measurement would be ± 0.016
(0.156 x 0.105 = 0.016)

This results in a range of 0.140 g/dL – 0.172 g/dL for the uncertainty calculation.

<u>To summarize, the measured uncertainty for blood alcohol analysis is ± 10.5 % to the 99.7 % confidence level</u>.

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, Medium and High Positive Controls

Note: Using all three positive controls results in an average %RSD that encompasses the entire calibration range.

Type B:

1. Glass pipettes used to measure volume of blood sample

2. NIST traceable reference solutions, purchased from Cerilliant

Table 2

EXAMPLE CALCULATION

Control	Low	Medium	High
mean	3.1	20.7	41.45
stddev	0.31	1.84	4.08
%CV	10.29	8.91	9.48

Average %CV = 29.04/3

= 9.68%

Average %RSD across the entire calibration range = 9.68%

Source of Uncertainty (Type A)	Value	Distribution	Divisor	Uncertainty
Average %RSD	9.68%	Normal	1	9.68%
Source of Uncertainty (Type B)	Value	Distribution	Divisor	Uncertainty
Volume of Sample	2.00	Rectangular	√3	1.15%
2.0 mL glass transfer pipette 2.0 mL ± 0.04 mL				
Cerilliant Standard Concentration	1.00%	Normal	2	0.50%
THC Standard Concentration 1.000 mg/mL ± 0.010 mg/mL				

Combined Uncertainty = $\sqrt{(9.68^2 + 1.15^2 + 0.50^2)} = 9.76\%$

n = 284

9.76% x k3 = 29.28%

THC Uncertainty to 99.7% confidence level = 29%

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 ± 29%
15 x 0.29 = 4.35

Calculated, the uncertainty of the measurement is ± 4 ng/ml

This results in a range of 11-19 ng/ml THC for the uncertainty declaration.

1.11.4 Summary of 2020 Alcohol/Drug Uncertainty Calculations

Thermo Ethyl Alcohol Uncertainty to 99.7% confidence level = 9.6%
Agilent Ethyl Alcohol Uncertainty to 99.7% confidence level = 9%
Butalbital Uncertainty to 99.7% confidence level = 19 %
Carisoprodol Uncertainty to 99.7% confidence level = 25%
Meprobamate Uncertainty to 99.7% confidence level = 43%
Phenobarbital Uncertainty to 99.7% confidence level = 38%
Alprazolam Uncertainty to 99.7% confidence level = 36%
Amphetamine Uncertainty to 99.7% confidence level = 22%
Benzoylecgonine Uncertainty to 99.7% confidence level = 23%
Diazepam Uncertainty to 99.7% confidence level = 20%
Methadone Uncertainty to 99.7% confidence level = 41%
Morphine Uncertainty to 99.7% confidence level = 24%
Nordiazepam Uncertainty to 99.7% confidence level = 28%
Temazepam Uncertainty to 99.7% confidence level = 18%
Tramadol Uncertainty to 99.7% confidence level = 33%
THC Uncertainty to 99.7% confidence level = 28%
THC-COOH Uncertainty to 99.7% confidence level = 19%

LCDOA Specific Drugs

Amphetamine Uncertainty to 99.7% confidence level = 21%
Benzoylecgonine Uncertainty to 99.7% confidence level = 14%
Buprenorphine Uncertainty to 99.7% confidence level = 19%
Cocaine Uncertainty to 99.7% confidence level = 9%
Codeine Uncertainty to 99.7% confidence level = 14%
Fentanyl Uncertainty to 99.7% confidence level = 16%
Hydrocodone Uncertainty to 99.7% confidence level = 16%
Hydromorphone Uncertainty to 99.7% confidence level = 15%
Morphine Uncertainty to 99.7% confidence level = 14%
Oxycodone Uncertainty to 99.7% confidence level = 13%
Oxymorphone Uncertainty to 99.7% confidence level = 15%

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
7. ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX D Toxicology Testing Discipline Example – Concentration of Ethanol in an Ante-Mortem Blood Specimen