ESTIMATION AND REPORTING OF MEASUREMENT UNCERTAINTY

The Laboratory employs policies and procedures for the estimation of measurement uncertainty. The evaluation process includes identification of the uncertainty components, and provides a reasonable estimation of the uncertainty of measurement.

Any adjustments or deviations from the procedures below must be approved by the QA Manager or Toxicology Laboratory Division (TLD) Commander/State Toxicologist, and appropriately documented in the relevant technical records.

6.1 POLICY

The Laboratory will evaluate the measurement uncertainty associated with quantitative results reported from test methods used in toxicological analysis. The following elements will be considered, and documented, for the estimation of measurement uncertainty (*see also 6.5.1*):

6.1.1 Statement defining the measurand

6.1.2 Statement of how traceability is established for the measurand

6.1.3 The equipment (e.g. measuring devices or instruments) used

6.1.4 All uncertainty components considered

6.1.5 All uncertainty components of significance and how they were evaluated

6.1.6 Data used to estimate repeatability or reproducibility

6.1.7 All calculations performed

6.1.8 The combined standard uncertainty, the coverage factor, the coverage probability and the resulting expanded uncertainty

6.1.9 The schedule to review and/or recalculate the measurement uncertainty

6.2 DEFINITIONS

All definitions are derived from the **VIM** (*see 6.5.8*).

6.2.1 Calibration Curve: Expression of the relation between indication and corresponding measured quantity value

6.2.2 Certified Reference Material (CRM): Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures

6.2.3 Combined Standard Measurement Uncertainty: Standard measurement uncertainty that is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model

6.2.4 Coverage Factor: Number larger than one by which a combined standard measurement uncertainty is multiplied to obtain an expanded measurement uncertainty

6.2.5 Coverage Interval: Interval containing the set of true quantity values of a measurand with a stated probability, based on the information available

6.2.6 Coverage Probability: Probability that the set of true quantity values of a measurand is contained within a specified coverage interval

6.2.7 Expanded Measurement Uncertainty: Product of a combined standard measurement uncertainty and a factor larger than the number one

6.2.8 Indication: Quantity value provided by a measuring instrument or measuring system

6.2.9 Measurand: Quantity intended to be measured

6.2.10 Measurement Bias: Estimate of a systematic measurement error

6.2.11 Measurement Error: Measured quantity value minus a reference quantity value

6.2.12 Measurement Precision: Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

6.2.13 Measurement Reproducibility: Measurement precision under reproducibility conditions of measurement

6.2.14 Measured Quantity Value: Quantity value representing a measurement result

6.2.15 Measurement Traceability (refers to Metrological Traceability): 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

6.2.16 Measurement Uncertainty: Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used

6.2.17 Random Measurement Error: Component of measurement error that in replicate measurements varies in an unpredictable manner

6.2.18 Reference Material (RM): Material, sufficiently homogenous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

6.2.19 Relative Standard Measurement Uncertainty: Standard measurement uncertainty divided by the absolute value of the measured quantity value

6.2.20 Reproducibility Condition of Measurement: Condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects

6.2.21 Systematic Measurement Error: Component of measurement error that in replicate measurements remains constant or varies in an predictable manner

6.2.22 Standard Measurement Uncertainty: Measurement uncertainty expressed as a standard deviation

6.2.23 Type A Evaluation of Measurement Uncertainty: Evaluation of a component of measurement uncertainty by a statistical analysis of measured quantity values obtained under defined measurement conditions

6.2.24 Type B Evaluation of Measurement Uncertainty: Evaluation of a component of measurement uncertainty determined by means other than a Type A evaluation of measurement uncertainty

6.2.25 Uncertainty Budget: Statement of a measurement uncertainty, of the components of that measured uncertainty, and of their calculation and combination

6.3 PROCEDURE

6.3.1 The measurand will be defined. The measurand is the concentration of the drug being quantified (e.g. concentration of THC in blood).

6.3.2 Determine how traceability is established for the measurand defined in 6.3.1. This may include documentation of RMs or CRMs and calibration of equipment used in testing. The following components establish traceability (not limited to):

- Use of a CRM (certificate of analysis from manufacturer states traceability to International Units)
- Use of piston pipettes calibrated by an approved external calibration provider that demonstrates traceability of equipment used in calibration to International Units
- Use of serialized, class-A volumetric glassware in preparation of standard solutions

6.3.3 The equipment and instrumentation used in performance of the test method will be considered in the estimation of measurement uncertainty. This includes, but is not limited to, calibrated piston pipettes, analytical balances, automatic diluters, volumetric flasks, gas or liquid chromatographs and mass spectrometers.

6.3.4 All uncertainty components considered in the estimation will be identified and evaluated to determine if they contribute significantly to the measurement uncertainty. A cause and effect diagram may be used for graphical representation of the uncertainty components and relationships.

6.3.5 Those components determined as significant will be included in the final uncertainty budget. The evaluation of significant components will be documented, and when any

components considered in 6.3.4 are not identified as significant and included in the uncertainty budget, the reason should be noted.

NOTE: Contribution to the estimated measurement uncertainty from components such as the preparation/addition of internal standard, the extraction process, and instrument acquisition may be accounted for by the reproducibility data (depending on factors such as the population size, *n,* and test method).

6.3.6 The source of data used in estimation of the repeatability or reproducibility components must be clearly identifiable. For example, if positive quality control data or validation data is used in reproducibility evaluation, the quality control tracking or validation spreadsheet should be included as supporting material. The associated batch records shall provide traceability information (e.g. testing analyst, date of testing, instrument identification, traceability).

6.3.7 All statistical formulas used and calculations performed are recorded, with reference to any supporting materials (e.g., published articles, reference tables).

6.3.8 The final components of the combined standard uncertainty (uncertainty budget), their individual contributions to the combined standard uncertainty and the formula used for the calculation of the combined standard uncertainty shall be outlined. The coverage factor and coverage probability applied to the combined standard uncertainty, as well as the resulting expanded uncertainty shall be defined. The reasoning for choosing the coverage probability (must be at least 95.45%, also referred to as approximately 95%) shall be clearly stated.

6.3.9 Once the estimation of measurement uncertainty has been completed, the Laboratory will determine a schedule for the re-evaluation and/or recalculation of the measurement uncertainty. The schedule will be defined, and may be based on a set time frame (e.g. annually) or based on other factors (e.g. changes to the test method, instrumentation, equipment or quality control procedures).

An outline of the measurement uncertainty evaluation procedure is found in Appendix A.

6.4 REPORTING

A statement of the estimated measurement uncertainty shall be included in the test report when it is relevant to the validity or application of the test results, when a customer requires, or when the uncertainty affects compliance to a specification (e.g. *per se*) limit.

6.4.1 Measurement uncertainty is reported for all quantitative blood and vitreous ethanol results. This is described in the *Policy on Reporting of Blood Alcohol Results (P46-1)*.

6.4.2 Measurement uncertainty is reported for all quantitative THC results.

6.4.3 Measurement uncertainty for quantitative results for compounds other than ethanol and THC is not included in the test report, but is available to the customer upon request. The customer will be notified (e.g. information on the TLD website and/or

comment on the test report) that measurement uncertainty for these quantitative results is available from the Laboratory.

6.4.4 When measurement uncertainty is reported for ethanol and THC, the value shall be reported in the test report or an attachment to the report, and shall be expressed as an expanded uncertainty and include the coverage probability.

6.4.5 When measurement uncertainty for compounds other than ethanol and THC is requested, the measurement uncertainty will be provided (using format described in 6.4.6 – 6.4.8) in writing (e.g. response letter) that clearly references the test report through its unique identifier.

6.4.6 The measurement result shall include the measurand quantity value, *y*, along with the associated expanded uncertainty, *U*, and this measurement result shall be reported as $y \pm U$ where *U* is consistent with the units of *y*.

6.4.7 The test result is truncated and reported to two significant digits. The calculated expanded uncertainty is rounded (normal, mathematical rules of rounding apply) and reported to two significant digits.

6.4.8 The measurement uncertainty calculations for blood and vitreous ethanol results and THC results will be verified by the analyst issuing the test report and at time of the technical and administrative review of the test report and case file.

6.4.9 The measurement uncertainty calculations for compounds other than ethanol and THC will be performed and reviewed by those personnel authorized by the TLD Commander/State Toxicologist. Documentation of the performance and review of the calculations will be retained in the case file.

6.4.10 Evaluation of measurement uncertainty for results reported from novel test methods or test methods that are not part of the laboratory's core test battery, are performed upon customer request.

6.4.11 All documentation related to measurement uncertainty evaluation, including data, calculations and documentation of review will be retained by the Quality Assurance (QA) Manager.

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APPENDIX A

The following is an outline of the evaluation of measurement uncertainty for quantitative results reported from the core battery of tests performed by the Laboratory.

I. Define the measurand:
The measurand is the concentration of drug in a blood sample

II. Traceability for the measurement is established through the use of:
Calibrated equipment (pipettes, glassware), with that calibration traceable to SI units of measurement
Use of CRMs from manufacturers that provide traceability to SI units of measurement

III. Equipment and instrumentation used:
Calibrated, adjustable piston pipettes
Volumetric glassware
Gas or liquid chromatographs, mass spectrometers, tandem mass spectrometers, headspace gas chromatographs

IV. All uncertainty components considered:
u(r) – experimental uncertainty arising from random effects
u(cal) – uncertainty in the preparation of working standard solution used to prepare calibrators
u(ctl) – uncertainty in the preparation of working control standard solution used to prepare positive controls
u(IS) – uncertainty in the preparation of the stock and/or working internal standard solutions
u(cur) – uncertainty arising from use of a linear least-squares regression curve
u(CRM) – uncertainty in the concentration of the CRM used in preparation of working standard and working control standard solutions
$u(R_m)$ – uncertainty associated with the evaluation of systematic error (bias)
u(sp) – uncertainty arising from the process of sample preparation
u(calprep) – uncertainty arising from the process of spiking calibrators with working standard and prepared dilutions of the working standard
u(ctlprep) – uncertainty arising from the process of spiking positive controls with working control standard and prepared dilutions of the working control standard
u(ISprep) – uncertainty arising from the addition of internal standard (IS) during sample preparation
u(inst) – uncertainty associated with the use of instrumentation for sample analysis (instrument precision)
u(environ) – uncertainty associated with the impact of environmental factors on the results

V. Description of how each component was evaluated and data used (see below)

VI. Calculations performed (described below):

u(r) = random error (reproducibility) uncertainty

Reproducibility uncertainty is obtained using statistics from all levels of positive quality controls (sourced from casework runs and/or validation and certification runs). For new or novel test methods, validation data may be used in evaluation of *u(r)*. Type A data. Assume normal distribution.

The control values for all lots are combined, and the mean and standard deviation are calculated for each control level (individual values outside ±3SD of the mean are considered outliers and are subsequently excluded).

Relative standard deviations are calculated and, when multiple positive control levels are used, pooled using the following equation, where *n* is the number of data points for the n_i control level and RSD_i is the relative standard deviation at that level:

$$u_r = RSD_{pooled} = \sqrt{\frac{(n_1 - 1) \times {RSD_1}^2 + (n_2 - 1) \times {RSD_2}^2 + (n_3 - 1) \times {RSD_3}^2}{(n_1 - 1) + (n_2 - 1) + (n_3 - 1)}}$$

u (cal) = uncertainty in the preparation of the working standard solution

Uncertainty in the preparation of working standard solution used to spike calibrators; includes purity of CRM (largest allowable from supplier), measured amount of CRM, thermal expansion coefficient, density of methanol or acetonitrile and standard tolerance of the density meter, total volume of solution prepared, tolerance of volumetric flask (maximum for respective volume) and SD from flask filling repeatability experiment. Repeatability term is determined by calculating the SD for repeated filling/weighing (n=10) of class A volumetric flasks with deionized water, using a five-place analytical balance (calibration traceable to SI units). Type A data. Assume normal distribution.

The following equation is used to calculate *u(cal)*:

$$u(cal) = \sqrt{\left(\frac{u(V_{CRM})}{V_{CRM}}\right)^2 + \left(\frac{u(VC_{CRM})}{VC_{CRM}}\right)^2 + \left(\frac{u(d_{MeOH})}{d_{MeOH}}\right)^2 + \left(\frac{u(V_{final})}{V_{final}}\right)^2}$$

The uncertainty in the measurement of CRM is calculated using the following equation:

$$u(V_{CRM}) = \sqrt{u(Pipette)^2 + u(Temp)^2}$$

$$u(Pipette) = \frac{volume\ measured * 0.03}{\sqrt{6}}$$

Maximum inaccuracy of calibrated piston pipette is 3%. Assume triangular distribution.
If a powdered reference material is weighed, uncertainty in the weighing of the solid reference material $u(m_{CRM})$ is used in place of *u(pipette)*. Assuming rectangular distribution, the uncertainty in the analytical balance (from manufacturer or calibration report) is divided by $\sqrt{3}$.

$$u(Temp) = \frac{0.00149 * 4 * volume\ measured}{\sqrt{3}}$$

Thermal expansion coefficient of methanol @20°C = 0.00149 /°C
Maximum environmental temperature variation = ±4°C
Assume rectangular distribution.

The uncertainty in the verified concentration of the CRM is calculated using the following equation:

$$u(VC_{CRM}) = \frac{purity\ of\ CRM}{\sqrt{3}}$$

Purity of CRM is from supplier's Certificate of Analysis (apply maximum allowable per specifications). For example, if listed purity is ≥97%, enter 0.03. Assume rectangular distribution.

Uncertainty in the density of methanol (or acetonitrile) is calculated using the following equation:

$$u(d_{MeOH}) = \frac{0.001 g/mL}{\sqrt{3}}$$

Density of methanol = 0.791 g/mL
Tolerance of a standard density meter = ±0.001g/mL
Assume rectangular distribution.

Uncertainty in the final volume of standard solution prepared is calculated using the following equation:

$$u(V_{final}) = \sqrt{u(Flask)^2 + u(Temp)^2 + u(Rep)^2}$$

$$u(Flask) = \frac{flask\ tolerance}{\sqrt{6}}$$

Tolerance of ASTM class A serialized volumetric flask @ 20°C (specific to volume)
Assume triangular distribution.

$$u(Temp) = \frac{0.00149 * 4 * flask\ volume}{\sqrt{3}}$$

Thermal expansion coefficient of methanol @20°C = 0.00149 /°C
Maximum environmental temperature variation = ±4°C

$$u(Rep) = SD\ from\ flask\ filling\ repeatability$$

Repeatability in filling the ASTM class A serialized volumetric flask to the final volume (n=10)

u (ctl) = uncertainty in the preparation of the working control standard solution

Same components and calculations as listed above for uncertainty in the preparation of the working standard solution. The working control standard is prepared using a different CRM than is used for the working standard or is prepared by a different individual.

u(IS) = uncertainty in the preparation of stock and/or working internal standard solution

Same components and calculations as listed above for uncertainty in the preparation of the working standard and working control standard. Working internal standard is added to all tubes in extraction, with quantification of target compounds based on the response ratio of target compound to internal standard. The preparation and use of internal standard can be accounted for in the reproducibility data, as it represents extractions performed by different analysts, data acquired on different instruments over the lifetime of the assay and different preparations (lot numbers) of stock and/or working internal standard.

u(cur) = uncertainty in the least squares linear regression curve

Uncertainty in the least-squares linear regression curve (with weighting specific to the assay, i.e. equal, 1/x, $1/x^2$) is evaluated using equation E3.5 from the *Eurachem/CITAC Guide, Quantifying Uncertainty in Analytical Measurement*. Calibrator target compound and internal standard responses from multiple test batches are used to generate an equal or weighted least-squares regression curve that is then used to quantify the corresponding positive controls. Type A data. Assume normal distribution.

The target compound area ratio for each calibrator is divided by its internal standard area ratio to calculate the response ratio for each calibrator and positive control.

The average response ratios for each calibrator level are calculated. The slope and y-intercept of the weighted linear least squares regression line are generated in Excel (for weighted regression lines, the *x* and *y* weighted averages are calculated prior to slope and y-intercept). Residuals are calculated for each calibrator from the line equation, using the corresponding response ratio.

The sum of the residual values is calculated using the following equation, where A_i is the response ratio for the *i* calibrator level, c_i is the target calibrator concentration for the *i* calibrator level, B_0 is the intercept of the regression curve, B_1 is the slope of the regression curve, and *n* is the number of calibrators in the calibration range:

$$S = \sqrt{\Sigma[A_i - (B_0 + B_1 \times c_i)]^2 \div n\text{-}2}$$

Squared deviations in *x* for each calibrator level are calculated by subtracting the average calibrator concentration from the individual target calibrator concentrations and squaring the results. The sum of the squared deviations in *x* is then calculated using the following formula, where c_i is the target calibrator concentration at the *i* level and *c* is the average calibrator concentration:

$$S_{xx} = \Sigma\ (c_i\text{-}c)^2$$

The weighted linear least squares regression line equation is used to calculate values from the individual response ratios for each of the positive controls. The average is then calculated for each set of control values.

The standard uncertainty at each positive control level is calculated using the following equation (individual terms are defined in Excel spreadsheet):

$$u(Ctl_{val}) = S/B_1 \sqrt{(1/p + 1/n + (c_0 - c)^2 \div S_{xx})}$$

The *relative* uncertainty for each positive control level is calculated for inclusion in the summary budget. The relative uncertainty is the standard uncertainty at the specified positive control level divided by the target concentration of that positive control level.

u(CRM) = uncertainty in the certified reference material

CRM (e.g. Cerilliant, Lipomed) certificates of analysis provide manufacturer uncertainty at k=2 (approx. 95%). Several lot numbers of CRMs produced by the approved reference material suppliers are referenced, and the largest listed uncertainty, or largest allowable uncertainty based on supplier specifications, is used. Manufacturer uncertainty incorporates components such as purity factor, material density, balance/weighing technique and the concentration is corrected for chromatographic purity, residual H_2O and residual solvents/inorganics. The value from the COA is divided by 2 to get the standard uncertainty (k=1), for inclusion in the summary budget. Type B data. Assume normal distribution.

u (R_m) = recovery uncertainty (bias)

For target compounds with a legal specification limit (blood ethanol and THC), recovery uncertainty (bias) is evaluated. Evaluation is performed as analysis of standard reference materials (SRM) replicates (NIST 0.08 g/100 mL ethanol) or a pooled whole blood control prepared to a THC target concentration of 5.0 ng/mL THC. Type A data. Assume normal distribution.

The method recovery is calculated as the ratio of the observed concentration to the reference concentration. The uncertainty for the NIST SRM is provided on the certificate of analysis. The uncertainty in the preparation of the pooled whole blood control is calculated. Statistical calculations are performed for the bias evaluation data using the Excel spreadsheet.

A significance test is applied to the data set(s), using the following equation: $$t = \frac{|1 - \bar{R}_m|}{u(\bar{R}_m)}$$

A coverage factor of k=3 is applied for the significance test. If the bias is found to be significant, the following equation is used to increase the standard uncertainty from method recovery to ensure that the final confidence interval includes the true value:

$$u(R_m) = \sqrt{(1 - R_m/k)^2 + u(R_m)^2}$$

NOTE: For ethanol uncertainty, a NIST SRM at 0.08 g/100mL was used to examine bias. Since a similar SRM does not exist for THC in blood, the preparation of the pooled whole blood control was used to estimate bias at the legal specification limit (5.0 ng/mL). Specific bias studies were not performed for compounds that do not have a current legal specification limit. Future bias studies may be performed to evaluate the effects of bias on results from other test methods, with the measurement uncertainty budget for the test method(s) updated accordingly.

u(sp) = uncertainty in the sample preparation

Calibrated piston pipettes are used to measure samples of blank blood and case specimen samples for use in drug testing. Calibrated automatic diluters are used to measure case specimens, calibrators and controls in blood ethanol testing. The maximum imprecision for calibrated piston pipettes is 2% (all volumes). The maximum imprecision for automatic diluters used in blood ethanol sample preparation is 0.2%. Pipetting of the sample (and blank blood used in preparation of calibrators/controls in drug testing) is the only contributor added for sample preparation, as other components (IS addition, extraction process) are accounted for by the reproducibility component. Type B data. Assume rectangular distribution (divide 0.02 or 0.002 by square root of 3).

u(calprep) = uncertainty in the spiking of calibrators with working standard and dilutions

This is reflected in the reproducibility data *u(r)* and in the *u(cur)* component, as these data sets include multiple sets of calibrators spiked by different analysts from different preparations (lot numbers) of working standard and analyzed on different instruments over time.

u(ISprep) = uncertainty in the addition of working internal standard to all members of the test batch at time of sample preparation

This is reflected in the reproducibility data, as the same amount of internal standard is added to all members of the test batch (except blank matrices). Quantification of target compounds is based on the response ratios of target compound to internal standard.

u(ctlprep) = uncertainty in the spiking of positive controls with working control standard and dilutions

This is reflected in the reproducibility data, which represents test batches extracted by all certified analysts, data acquired on multiple instruments and different preparations (lot numbers) of working control standard.

u(inst) = uncertainty in the use of instrumentation

This includes sample injection, instrument variability and sensitivity and use of acquisition software. This is reflected in the reproducibility data, which represents test batches acquired on multiple instruments over time.

u(environ) = uncertainty due to effects of environmental conditions

This is reflected in the reproducibility data, which represents test batches extracted by all certified analysts and data acquired on multiple instruments, over time.

VII. Combined standard uncertainty, coverage factor, coverage probability and resulting expanded uncertainty

u(comb) combined standard uncertainty

The root sum of squares method was used to calculate the combined standard uncertainty from the significant components listed above. Note that the $u(R_m)$ – recovery uncertainty (bias) component is only included in evaluation of measurement uncertainty for blood ethanol and THC. The *u(Ctl)* component is not included in evaluation of blood ethanol uncertainty of measurement, as CRMs from an approved, external supplier are used as positive controls.

The following formula is used to calculate the combined standard uncertainty:

$$u_{comb} = \sqrt{(r)^2 + (cur)^2 + (CRM)^2 + (Ctl)^2 + (Cal)^2 + (SP)^2}$$

The expanded uncertainty is calculated by multiplying the combined standard uncertainty by the coverage factor, *k*, to obtain the desired coverage probability for the coverage interval. For all drugs other than blood ethanol and THC, a coverage probability of 95.45% (or approximately 95%) is applied, with a coverage factor of 2. For blood ethanol and THC, a coverage probability of 99.7% is applied, with a coverage factor of 3.

VIII. Schedule to review and/or recalculate the measurement uncertainty

The measurement uncertainty procedure and uncertainty budgets will be reviewed annually to determine whether reevaluation and/or recalculation are required. Examples of when reevaluation may be warranted include changes to a test method (e.g. extraction, instrumentation, equipment, quality control procedures) or changes in critical reagent/material or equipment calibration suppliers. Review will be documented in an inter-office communication (IOC) to the TLD Commander/State Toxicologist or QA Manager. Reevaluation documentation (data, calculations) will be retained by the QA Manager, filed in the measurement uncertainty records for the specific test method.

For infrequently performed or novel test methods, as data is limited, the measurement uncertainty evaluation may not include all components listed above.

LIST OF CHANGES

Revision Date	Change	Page Number
1/6/16	Procedure approved by the Washington State Toxicologist. See DRA dated 1/6/16.	All