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Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology

ASB ACADEMY STANDARDS BOARD

Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology

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This document may be downloaded from: www.aafs.org/academy-standards-board

Foreword

This document was developed to provide the minimum requirements for evaluating measurement uncertainty for quantitative measurements in forensic toxicology testing laboratories and calibration of breath alcohol measuring instruments by breath alcohol programs. Measurement uncertainty is required to ensure confidence, reliability, and proper interpretation of test or calibration results. It is also one of the components used to establish metrological traceability.

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This document was revised, prepared, and finalized as a standard by the Toxicology Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aafs.org or 401 N 21st Street, Colorado Springs, CO 80904.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

ASB procedures are publicly available, free of cost, at www.aafs.org/academy-standards-board.

Keywords: measurement uncertainty; forensic toxicology; breath alcohol instrument calibration

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Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology

1 Scope

This document provides minimum requirements for evaluating measurement uncertainty for quantitative forensic toxicology testing activities as well as calibration of breath alcohol measuring instruments. Specifically, it is intended for the subdisciplines of postmortem forensic toxicology, human performance toxicology (e.g., drug-facilitated crimes and driving-under-the-influence of alcohol or drugs), non-regulated employment drug testing, court-ordered toxicology (e.g., probation and parole, drug courts, child services), and general forensic toxicology (non-lethal poisonings or intoxications) as well as calibration of breath alcohol measuring instruments.

It does not address evaluating measurement uncertainty for breath alcohol subject testing. Nor does it address uncertainty or performance measures for qualitative forensic toxicology testing activities.

2 Normative References

The following references are documents that are indispensable for the application of the standard. The latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 017, *Standard for Metrological Traceability in Forensic Toxicology* [a]

ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology* [a]

ANSI/ASB Standard 053, *Standard for Reporting in Forensic Toxicology* [a]

ANSI/ASB Standard 054, *Standard for a Quality Control Program in Forensic Toxicology Laboratories* [a]

ANSI/ASB Standard 055, *Standard for Breath Alcohol Measuring Instrument Calibration* [a]

3 Terms and Definitions

For purposes of this document, the following definitions and acronyms apply.

3.1
analytical run
"batch"
Set of standards, controls, and/or case samples that are contemporaneously prepared and/or analyzed in a particular sequence

3.2
bias, analytical
Estimate of systematic measurement error, calculated as the difference between the mean of several measurements under identical conditions to a known "true" value

[a] Available from: https://www.aafs.org/academy-standards-board

3.3
calibration [b(Mod)]
Operation that, under specified conditions, establishes a relationship between the quantity value and corresponding indications

3.4
calibrator [b]
Measurement standard used in calibration

3.5
certified reference material [c]
CRM
Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

3.6
control
Material of known composition that is analyzed along with unknown samples(s) in order to evaluate the performance of an analytical procedure

3.7
limit of detection
LOD
Estimate of the lowest concentration of an analyte in a sample that can be reliably differentiated from blank matrix and identified by the analytical method

3.8
lower limit of quantitation
LLOQ
Estimate of the lowest concentration of an analyte in a sample that can be reliably measured with acceptable bias and precision

3.9
measurand [b]
Quantity intended to be measured

3.10
measurement standard [b(Mod)]
Reference, with a stated value and associated measurement uncertainty, used to calibrate or verify measuring instruments or measuring systems

[b] Joint Committee for Guides in Metrology (JCGM), International vocabulary of metrology – Basic and general concepts and associated terms (VIM), 3rd ed. (Sèvres, France)
[c] International Organization for Standardization (ISO), ISO Guide 30:2015 Reference Materials – Selected Terms and Definitions (Geneva, Switzerland)

3.11
metrological traceability [b]
(measurement 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

3.12
precision [b](Mod)
Measure of the closeness of agreement between a series of measurements obtained by replicate measurements on the same or similar samples

3.13
repeatability [b](Mod)
Measurement precision under a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions, same location, and replicate measurements on the same or similar objects over a short period of time

3.14
reproducibility [b](Mod)
Measurement precision under a set of conditions that includes different locations, operators, measuring system, and replicate measurements on the same or similar objects

3.15
type A evaluation (of uncertainty)
Method of evaluation of uncertainty by the statistical analysis of series of observations (e.g., relative standard deviation of a historical data set of control results)

3.16
type B evaluation (of uncertainty)
Method of evaluation of uncertainty by means other than the statistical analysis of series of observations (e.g., obtaining the uncertainty associated with a CRM from its certificate of analysis)

4 Background

Quantitative values obtained from measurement processes have an expected variability. Repeated measurements will result in different values each time a measurement is made, provided the measuring system has sufficient resolution to allow those differences to be seen. Each time a measurement is made, the measured value depends on numerous factors, including the setup and capability of the measuring system, the exact measurement method (procedure), and the person performing the measurement.

Measurement Uncertainty (MU) is an estimate of the potential variability of a measurement based on the information known about the measurand and the measurement method. The measurement may be part of the test, a calibration method, or the final reported test or calibration result. "Measurement uncertainty does not imply doubt about the validity of a measurement; on the contrary, knowledge of the uncertainty implies increased confidence in the validity of the measurement result." [d]

[d] SLR Ellison and A Williams (Eds). Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (QUAM: 2012 P1) Available from: http://www.eurachem.org/index.php/publications/guides

Stakeholders require tests and calibrations to be reliable, accurate, and comparable. MU is an important parameter describing the confidence and limitations of measurement results. Comparing quantitative test or calibration results between testing laboratories or evaluating quantitative results in relation to a legal specification or requirement necessitates knowledge of the MU.

The National Institute for Standards and Technology (NIST) has developed an 8-step process for evaluating and reporting MU (Figure 1).[e] This framework established by NIST conforms to the principles set forth in the Joint Committee for Guides in Metrology (JCGM) Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement (GUM[f]) and is a helpful reference.

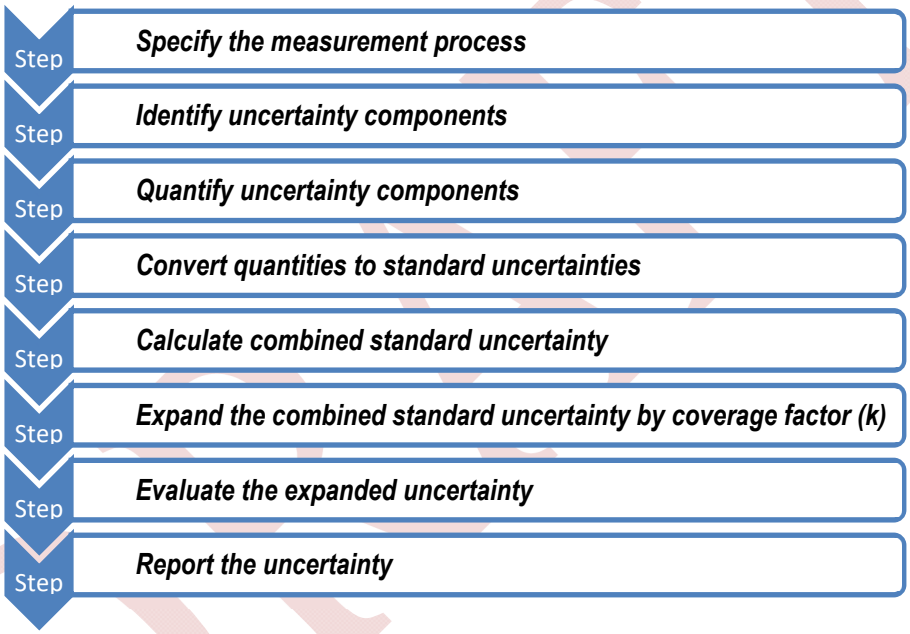

Figure 1—The NIST 8-Step Process for Evaluating and Reporting Measurement Uncertainty

[e] National Institute of Standards and Technology, SOP 29-Standard Operating Procedure for the Assignment of Uncertainty (April 2021). Available from: https://www.nist.gov/system/files/documents/2019/05/13/sop-29-assignment-of-uncertainty-20190506.pdf

[f] Joint Committee for Guides in Metrology (JCGM) Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement (GUM) (GUM 1995 with minor corrections) (Sevres, France: International Bureau of Weights and Measures [BIPM]-JCGM 100], September 2008. Available from: http://bipm.org/en/publications/guides/gum.html

5 Requirements for Measurement Uncertainty for Quantitative Determinations

5.1 General Requirements

5.1.1 Testing laboratories and breath alcohol programs shall have and apply procedures for evaluating MU for test methods that produce a quantitative test result and for methods used to calibrate breath alcohol measuring instruments.

5.1.2 Records of MU evaluations shall be maintained.

5.1.3 MU shall be evaluated for each measurement process and is specific to the measurement process. This includes, but is not limited to:

5.1.3.1 Each calibration method shall be evaluated separately.

5.1.3.2 Each combination of analyte, extraction, and analytical technique shall be evaluated separately.

NOTE 1: MU specific to each measurement process means not using the largest evaluated MU for more than one analyte within a method or one analyte across methods.

NOTE 2: Statistical data evaluation may indicate a need to evaluate different matrices separately.

5.1.4 Test and calibration methods for which the MU is evaluated shall meet the minimum requirements set forth in:

a) ANSI/ASB Standard 017, *Standard for Metrological Traceability in Forensic Toxicology*.

b) ANSI/ASB Standard 036, *Standard Practices for Method Validation in Forensic Toxicology.*

c) ANSI/ASB Standard 054, *Standard for a Quality Control Program in Forensic Toxicology Laboratories.*

d) ANSI/ASB Standard 055, *Standard for Breath Alcohol Measuring Instrument Calibration.*

5.2 Step 1—Specify the Measurement Process

The measurand shall be defined.

NOTE: This can be a written statement, a visual diagram, and/or a mathematical expression. Be specific when defining the measurand.

EXAMPLES:

Testing of biological samples
Concentration of ethanol (g/100mL) in antemortem whole blood using GC-FID
Concentration of oxycodone (mg/kg) in a sample of liver homogenate using LC-MS/MS

Calibration of breath alcohol measuring instruments
Calibration of XYZ model breath alcohol measuring instrument using dry gas certified reference material

5.3 Step 2—Identify Uncertainty Components

Minimum method components that shall be considered, as applicable, in an evaluation of MU include:

a) certified reference material(s) and calibrations of equipment used to establish metrological traceability;

b) data from the measurement process (i.e., repeatability, reproducibility or from intermediate measurement conditions);

c) human factors (e.g., multiple personnel performing the same measurement method, experience, training);

d) sampling conducted during the measurement method;

e) sample preparation; and

f) environmental conditions during the measurement process.

5.4 Step 3—Quantify Uncertainty Components

5.4.1 *General*

5.4.1.1 Uncertainty components shall be quantified.

5.4.1.2 No fewer than three significant figures shall be carried through all calculations to ensure reporting requirements can be met.

5.4.1.3 The method of evaluation, Type A or Type B, shall be determined for each component.

NOTE 1: It is most common to use a mixture of the two methods, where some uncertainty components are quantified using a Type A method of evaluation and some uncertainty components are quantified using a Type B method of evaluation.

NOTE 2: Double counting of a component will result in overestimating the measurement uncertainty.

5.4.2 *Minimum Requirement(s) for Type A Evaluations*

5.4.2.1 General

Testing laboratories and breath alcohol programs shall specify in their procedure the source(s) of the Type A data to be used.

5.4.2.2 Testing Laboratories

5.4.2.2.1 Selection of Type A Data

5.4.2.2.1.1 Validation data may initially be used to evaluate one or more specific Type A uncertainty components.

5.4.2.2.1.2 Control data shall be used for the Type A uncertainty component after validation and implementation.

5.4.2.2.1.3 Proficiency test data may also be used for a Type A uncertainty component; however, if used, the test(s) shall have established metrological traceability.

NOTE: A consensus result does not establish metrological traceability.

5.4.2.2.1.4 Data used in Type A evaluations shall:

a) be representative of the measurand that will be tested;

b) be representative of the range (e.g., matrix, detector response over the expected concentration range) of the measurements made;

c) be representative of the data generated during ongoing analysis by personnel who have demonstrated competence; and

d) be evaluated according to the size and distribution of the statistical sample.

NOTE: Approaches to selecting Type A Data include, but are not limited to:

— *using control data generated since method implementation;*

— *using a laboratory-specified number of control data points from the most recent analyses; or*

— *using control data from only the current analytical batch in non-routine analyses with limited data points.*

5.4.2.2.2 Calculation of the Quantity Value for Type A Data

5.4.2.2.2.1 The standard deviation or relative standard deviation shall be calculated using data for each Type A uncertainty component.

NOTE 1: Method performance is typically represented by measurements of control samples taken over multiple batches, each with different calibrations.

NOTE 2: If multiple replicates of a control level are available per batch, the data from all replicates may be included when calculating the standard deviation or relative standard deviation. Including all data in the standard deviation calculation will bias the standard uncertainty slightly if the data exhibits any batch-to-batch variation but mitigates the need for more complex standard deviation calculations. This would provide an assessment of the Type A uncertainty that is either on target or conservative (i.e., overestimated) for the reported specimen value.

NOTE 3: If needed, other statistical methods, such as the ANOVA method or random subsampling of the data to select a single instance from each batch, can be used to correct this bias.

5.4.2.2.2.1.1 When the result to be reported for a specimen is either an individual measured value or the average of multiple measured values from a single instrumental batch, the standard deviation or the relative standard deviation shall be used as the Type A standard uncertainty for the reported specimen value.

5.4.2.2.2.1.2 When the result to be reported for a specimen is the average of measured values from multiple instrumental batches, the standard deviation or the relative standard deviation divided by the square root of the number of instrumental batches used when averaging the specimen data shall be used (i.e., standard deviation of the mean of multiple batches) as the Type A standard uncertainty for the reported specimen values.

5.4.2.2.2.2 Multiple Controls within the Same Method

Testing laboratories shall evaluate variance of control data (e.g., perform a statistical F-test).

5.4.2.2.2.2.1 If consistent variance is demonstrated, testing laboratories shall:

a) combine data from all controls analyzed; or

b) select data from one specified control (e.g., a control at or near a legal specification).

5.4.2.2.2.2.2 If consistent variance is not demonstrated, testing laboratories shall:

a) utilize the Type A data from the control producing the largest variance; or

b) perform an in-depth evaluation to determine where the variation change occurs and establish an appropriate uncertainty to report for each range.

5.4.2.2.2.3 Multiple Instruments and/or Laboratories

To calculate a single MU by combining data from multiple instruments and/or in multiple laboratories, control acceptance and reporting criteria shall be the same across all instruments and laboratories.

5.4.2.3 Calibration of Breath Alcohol Measuring Instruments

5.4.2.3.1 Selection of Type A Data

5.4.2.3.1.1 Validation data may initially be used to evaluate one or more specific Type A uncertainty components.

5.4.2.3.1.2 Reference material data generated during calibrations shall be used for the Type A uncertainty component after validation and implementation. Reference material data generated during control testing may be used in addition to that generated during calibrations.

5.4.2.3.1.3 Proficiency test data may also be used for a Type A uncertainty component; however, the test shall have established metrological traceability if used.

NOTE: A consensus result does not establish metrological traceability.

5.4.2.3.1.4 Data used in Type A evaluations shall:

a) be representative of the measurand that will be calibrated;

b) be representative of the range of the measurements made;

c) be representative of the data generated during ongoing calibrations performed by personnel who have demonstrated competence; and

d) be evaluated according to the size and distribution of the statistical sample.

NOTE: Approaches to selecting Type A data include, but are not limited to:

- *using reference material data generated since method implementation;*
- *using a breath alcohol program specified number of reference material data points from the most recent calibrations; or*
- *using reference material data from only the current calibration.*

5.4.2.3.2 Calculation of the Quantity Value for Type A Data

5.4.2.3.2.1 The standard deviation or relative standard deviation shall be calculated using data for each identified Type A uncertainty component.

5.4.2.3.2.2 Calibration Method (Multiple Measurement Standards)

Breath alcohol programs shall evaluate the variance between measurement standard data by performing a statistical test (e.g., perform a statistical F-test).

5.4.2.3.2.2.1 If a consistent variance is demonstrated, breath alcohol programs shall:

a) combine data from all measurement standards analyzed to calculate a single MU;

b) select data from one specified measurement standard (e.g., a concentration at or near a legal specification); or

c) calculate the quantity value for Type A data at each measurement standard concentration.

5.4.2.3.2.2.2 If a consistent variance is not demonstrated, breath alcohol programs shall:

a) utilize the Type A data from the measurement standard producing the largest variance;

b) perform an in-depth evaluation to determine where the variance changes occur across the calibration range and establish an appropriate uncertainty to report based on where these variance changes occur; or

c) calculate the MU at each measurement standard concentration.

5.4.2.3.2.3 Multiple Instruments

The calibration method and instrument make/model shall be the same to calculate MU by combining data from multiple breath alcohol measuring instruments.

5.4.3 *Minimum Requirements for Type B Evaluations*

5.4.3.1 Components requiring a Type B evaluation may include uncertainty associated with a certified reference material, uncertainty of a reference material, and/or uncertainty from equipment calibration (e.g., balance, volumetric flask, pipette, barometer, or thermometer).

5.4.3.2 When considering which components to include in the Type B evaluations, testing laboratories and breath alcohol programs shall:

a) consider all components that are not accounted for in a Type A evaluation;

b) ensure components are evaluated according to the assumed distribution of the quantity value; and

c) account for all identified and significant systematic bias (see 5.6.2).

5.4.4 *Establishing a quantity value for Type B evaluations*

5.4.4.1 For component(s) used in the preparation of a calibrator or measurement standard, the components shall be quantified individually or as a group.

5.4.4.1.1 If evaluating uncertainty over the full calibration range, testing laboratories and breath alcohol programs shall use the largest standard deviation calculated.

5.4.4.1.2 If evaluating the uncertainty for multiple concentration ranges, testing laboratories and breath alcohol programs shall use the largest standard deviation calculated for each concentration range, respectively.

5.4.4.1.3 If evaluating the uncertainty at each calibrator or measurement standard concentration separately, testing laboratories and breath alcohol programs shall use the value for the applicable calibrator or measurement standard.

NOTE 1: If the test or calibration method includes the preparation of multiple calibrators or measurement standards, the individual components may be quantified individually across all calibrator concentrations (e.g., a single component quantity value can be used for the pipette uncertainty that adequately covers the pipettes used to prepare all calibrator concentrations) and then 5.4.4.1.1 or 5.4.4.1.2 above may be applied. Alternatively, the components may be quantified as a group for each calibrator concentration and then 5.4.4.1.1 through 5.4.4.1.3 applied.

NOTE 2: Depending on the measurement process, these components related to calibrator preparation, typically requiring a Type B evaluation, may be accounted for by ongoing control data (Type A).

5.5 Step 4—Convert Quantities to Standard Uncertainties

5.5.1 *General*

The testing laboratory or breath alcohol program shall quantify all uncertainty components as a standard uncertainty of the quantity values and in the same measurement unit or in a measurement unit relative to the quantity values.

5.5.2 *Type A Evaluations*

5.5.2.1 If not already presented as a standard uncertainty, the quantity shall be divided by the appropriate coverage factor (k) to convert to a standard uncertainty.

5.5.3 *Type B Evaluations*

5.5.3.1 If not reported by the manufacturer as a standard uncertainty, the testing laboratory or breath alcohol program shall use the appropriate probability density function for the component to compute one standard deviation or relative standard deviation associated with the specified distribution.

5.5.3.2 If reported by the manufacturer as an expanded uncertainty, the testing laboratory or breath alcohol program shall divide by the appropriate coverage factor (*k*) to arrive at a standard uncertainty.

5.6 Step 5—Calculate the Combined Standard Uncertainty

5.6.1 *General*

The testing laboratory or breath alcohol program shall calculate the combined standard uncertainty using the uncertainty contributors' quantity values, utilizing the root sum of the squares formula or the Monte Carlo[g] method.

5.6.1.1 A justification shall be documented if any uncertainty component is excluded from the combined standard uncertainty.

5.6.2 *Evaluation of Bias* [h]

5.6.2.1 Measurement accuracy encompasses both precision and bias. A measurement is more accurate when it has less bias and greater precision. The GUM states, "It is assumed that the result of a measurement has been corrected for all recognized significant systematic effects and that every effort has been made to identify such effects." An evaluation of bias may not always be possible as one or more controls prepared with metrological traceability, having a known reference value and uncertainty, is required to evaluate bias.

5.6.2.2 Bias evaluation shall be performed whenever possible.

5.6.2.3 The general approach to bias evaluation shall:

a) Determine if bias is present by comparing measurement standard or control data to reference values with established metrological traceability;

b) Calculate the combined uncertainty without considering the relevant bias; and

c) Compare the bias with the combined standard uncertainty.

1) Where the bias is less than the combined standard uncertainty, bias$<u_c$, the bias is viewed as insignificant and may be neglected or included as a component in the uncertainty evaluation.

2) Where the bias is greater than or equal to the combined standard uncertainty, bias$\geq u_c$, it is considered significant and additional action shall be taken; see 5.6.2.4 and 5.6.2.5.

5.6.2.4 Testing laboratories shall address significant bias in one of the following ways:

[g] Joint Committee for Guides in Metrology (JCGM) Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement (GUM)-Supplement 1-Propagation of distributions using a Monte Carlo Method (Sevres, France: International Bureau of Weights and Measures [BIPM]-JCGM 101:2008], September 2008. Available from: https://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf

[h] Section 3.2.5 of NIST SOP 29 (2019)

a) modify the method to reduce the bias until it is no longer significant and the expanded uncertainty of the method remains fit for purpose;

b) correct the measurement result for the bias, including the uncertainty of the correction in the evaluation of uncertainty. Both the observed measurement result and the corrected measurement result with the MU shall be reported;

c) report the measurement result and the expanded MU with bias included; or

d) report the observed measurement result, the MU, and the bias.

5.6.2.5 Breath alcohol programs shall address significant bias in one of the following ways:

a) modify the method to reduce the bias until it is no longer significant and the expanded uncertainty of the method remains fit for purpose;

b) report the measurement result and the expanded MU with bias included; or

c) report the observed measurement result, the MU, and the bias.

5.7 Step 6—Calculate the Expanded Uncertainty

5.7.1 A coverage factor (*k*) shall be determined using a Student's *t*-distribution based on the degrees of freedom (n-1) to provide the desired confidence level.

5.7.2 The minimum coverage probability for all quantitative test results and calibration results shall be 95.45 %.

5.8 Step 7—Evaluate the Expanded Uncertainty

5.8.1 A determination of whether the calculated measurement uncertainty is acceptable shall be made by the testing laboratory or breath alcohol program.

5.8.2 The evaluation of acceptance, as applicable, shall consider:

a) stakeholder interests;

b) legal requirements;

c) the relationship between the reported test or calibration quantitative value and the expanded MU; particular consideration shall be taken around the LLOQ/LOD; and

EXAMPLE: An expanded MU of 0.01 ng/mL for a method with an LLOQ of 0.01 ng/mL would prompt the testing laboratory or breath alcohol program to reevaluate the LLOQ.

d) the relationship between the control limits for the method and the expanded measurement uncertainty.

EXAMPLE: Control limits of ± 20 % for a method with expanded MU of 10 % (95.45 % coverage probability). For any single analytical batch, this control limit would allow a variation of up to 20 % which exceeds the

stated expanded MU for the method and would prompt the testing laboratory or breath alcohol program to reevaluate the control limits.

5.9 Step 8—Report the Expanded Uncertainty

5.9.1 For testing laboratories, MU reporting shall be in accordance with ANSI/ASB Standard 053, *Standard for Reporting in Forensic Toxicology*.

5.9.2 For breath alcohol programs, the MU shall be reported as part of the calibration result.

5.9.3 When the MU is reported:

5.9.3.1 For testing laboratories, the MU shall be reported as an expanded uncertainty and include the coverage probability.

5.9.3.2 For breath alcohol programs, the MU shall be reported as an expanded uncertainty and include the coverage factor, *k*, and the coverage probability.

5.9.3.3 The measurement result shall include the measured quantity value, y, along with the associated expanded uncertainty, U. It should be reported as y ± U, where U is consistent with the units of y. Specific applications may warrant using a different format than y ± U.

5.9.3.4 The expanded uncertainty should be reported to at most 2 significant figures unless the testing laboratory or breath alcohol program has a documented rationale to report beyond 2 significant figures.

5.9.3.5 Rules for rounding the expanded uncertainty shall be defined by the testing laboratory or breath alcohol program.

5.9.3.6 The rounded expanded uncertainty shall be reported using the same number of decimal places as the measurement result unless a legal specification specifies how the result will be reported. Rules for rounding or truncating the measurement result shall be defined by the testing laboratory or breath alcohol program.

5.9.3.7 Testing laboratories shall report the respective measurement uncertainty for each analyte within a method.

NOTE: Combining the MU across multiple analytes or methods would lead to an overestimation of the MU, which does not meet the intent of a measurement uncertainty evaluation.

5.9.3.8 For testing laboratories, if a significant bias is identified and the action taken is as described in 5.6.2.3 b) or c), this shall be clearly communicated.

6 Periodic Evaluation of Measurement Uncertainty

6.1 The testing laboratory or breath alcohol program shall set the interval for reviewing and recalculating a method's MU and shall retain records supporting the decision.

6.2 For both Type A and Type B uncertainty components included in the MU calculation, the decision shall consider:

a) the frequency with which one of the components changes;

b) the frequency with which the testing or calibration method is performed;

c) the magnitude of a change in a component in relationship to the calculated MU;

d) subsequent sources of Type A data (e.g., changes to personnel, additional instrumentation);

e) a change in the measurement process; and

f) any testing laboratory or breath alcohol program administrative decision such as a set time interval.

6.3 Any recalculation of the measurement uncertainty shall meet all requirements of this standard.

Annex A
(informative)

Concentration of Ethanol in an Ante-mortem Blood Specimen[i]

Test Method Information

Multiple personnel were trained and qualified to use the laboratory's method to determine ethanol concentration in ante-mortem blood specimens. All personnel use the same equipment for this test method. This includes a pipette diluter that delivers the specified sample volume together with a specified volume of aqueous internal standard.

The test method relies on dual-column gas chromatography with two flame ionization detectors. The quantitative measurement is determined from one of the two columns. Samples are introduced to the gas chromatograph via a headspace autosampler.

Calibrators are used to generate a calibration curve with each analytical batch. They are certified reference materials (CRMs) and span the reportable concentration range (e.g., 0.020 g/dL to 0.400 g/dL). The CRMs are not altered before use (i.e., not diluted). Method validation indicated that the proper calibration model was an unweighted linear regression.

Measurement assurance is achieved through the use of control (QC) samples. These include a quantitative blood matrix control prepared by the laboratory at approximately 0.080 g/dL and CRMs at low, medium, and high concentrations (obtained from a different supplier than the CRMs used as calibrators). As with the CRMs used as calibrators, those used as QC samples are not altered before use. Consistent variance (homoscedasticity) was observed between all controls.

Test specimens are analyzed in two separate batches. The average of the two measurement results is reported; however, the procedure requires that the individual measurements be no more than 5% from the average or the analyses are repeated.

Calibrators, QC samples, and test samples are aliquoted in one instance using the same equipment.

Metrological Traceability

The traceability of this measurement process is established through the calibrators used to generate the calibration curve on the measuring system and through the calibration of other equipment used in the measurement process.

All CRMs have been purchased from a Reference Material Producer that meets the ANSI/ASB Standard 017, *Standard for Metrological Traceability in Forensic Toxicology*.

The external calibration of the pipette diluter is performed by calibration laboratories that meet the ANSI/ASB Standard 017, *Standard for Metrological Traceability in Forensic Toxicology*.

[i] An evaluation of measurement uncertainty is specific to the metrological traceability established for the measurement, the measurement assurance processes in place, the laboratory test method, the laboratory facility, etc. Therefore, the following is only an example for evaluation and revision by a laboratory after considering the elements specific to that laboratory.

Measurement Assurance

The laboratory prepared the quantitative blood matrix control to a concentration of approximately 0.080 g/dL. It is made in a large batch, packaged, and stored in a manner that provides a long shelf-life for the control. The expected concentration is determined in-house through repeat measurements.

The CRMs used for QC samples at low, medium, and high concentrations were purchased from a supplier different from the CRMs used as calibrators.

The QC samples are used to ensure the validity of the test method across the concentration range. The CRM QC samples are also used to verify the calibration curve and to evaluate the method's bias on an ongoing basis.

Step 1—Specify the measurement process

As a written statement:

"The Concentration of Ethanol in Ante-Mortem Blood using [the validated laboratory procedure]"

Step 2—Identify uncertainty components

The following list of possible contributors to the uncertainty in this method was identified by the laboratory:

<u>Personnel</u>

- Inter-personnel variation in sample preparation and measurements
- Training
- Experience

<u>Calibrators</u>

- CRM –uncertainty in the stated reference value
- Matrices of calibrators and test specimens

<u>Control Samples</u>

- CRM – second source; uncertainty in the stated reference value
- Matrix control – stability

<u>Internal Standard Preparation</u>

- Components:
  - NaCl – reagent grade

- n-propanol – reagent grade
- Concentration – equipment used to prepare (balance, volumetric flask)

Preparation of Aliquots of Calibrators, Control Samples and Measurand

- Homogenization
  - Test Specimens – mixing
  - Matrix control – mixing
- Temperature
  - All calibrators, control samples, and the test specimens are brought to room temperature
    - Variation in the time allowed to reach room temperature
    - Variation in room temperature at different times of year
- Pipette diluter
  - Volume of sample and volume of internal standard
    - Calibration uncertainty or laboratory specification to verify calibration status
- Headspace vials
  - Crimping action
  - Material of vial and stopper
- Time between replicate sampling of test specimens

Analysis

- Instrument parameter settings (e.g., oven temperature(s), gas flow, split ratio, aging of the chromatographic column, autosampler syringe, autosampler precision, headspace equilibration time, headspace equilibration temperature)
- Interference from the matrix
- Interference from reagents
- Interference from other compounds
- Stability of sample(s) from preparation through analysis
- Instrument precision

— Systematic instrumental variation within an analytical batch

Data Processing

— Calibration model

— Integration parameters

— Processing algorithms

NOTE: This list of uncertainty components to be considered could also be compiled into a fishbone diagram or any other format of the laboratory's choosing.

NOTE: A laboratory may identify different uncertainty components when evaluating their specific measurement process.

Step 3—Quantify uncertainty components

The laboratory has existing data from the measurement process.

— The calibration model was determined during method validation and was shown using a statistical test to have consistent variance across the linear range. Therefore, the laboratory will evaluate a single measurement uncertainty to represent the entire reportable concentration range.

— Each analytical batch includes one or more independently prepared samples of the blood matrix control. This blood matrix QC sample is prepared to have an ethanol concentration of approximately 0.080 g/dL. All personnel have made measurements using this blood matrix QC sample (across multiple lots). Pre-defined criteria for acceptable performance are based on historical data across multiple lots from the last 2 years. To date, the laboratory has had more than 100 measurements of the blood matrix QC sample since validation.

— The laboratory also has data from three certified reference materials that were used as control samples. The ethanol concentration of the CRM QC samples spans the reportable concentration range. The primary use of the CRM QC samples is to evaluate bias in the measurement method, but these samples also provide additional evaluation of several uncertainty components.

Table A.1 shows the individual uncertainty components and how they will be evaluated.

531 **Table A.1—Method of Evaluation of Uncertainty Components**

Uncertainty Component	Method of Evaluation
Personnel	
Inter-personnel variation	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Training	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Experience	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Calibrators	
CRM – uncertainty in the stated reference value	Type B Evaluation
Matrices of calibrators and test specimens	Initially evaluated during method validation, it was determined to be insignificant and, therefore, not included in the uncertainty evaluation.
Control Samples	
CRM – second source; uncertainty in the stated reference value	The primary use is to evaluate bias. The bias evaluation will be done after the calculation of combined standard uncertainty.
Matrix control - stability	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Internal Standard Preparation	
Components: NaCl – reagent grade n-propanol – reagent grade	The measurement result will only be impacted by the volume of the internal standard added to each sample (i.e., variation due to pipette diluter).
Concentration- equipment used to prepare (balance, volumetric flask)	Procedural requirement to use the same lot of Internal Standard for all samples in an analytical batch. The measurement result will only be impacted by variation in the volume of the internal standard added to each sample (i.e., variation due to pipette diluter).
Preparation of aliquots of Calibrators, Control Samples, and Test Specimens	
Homogenization – mixing	Initially evaluated during method validation, it was determined to be significant; therefore, it was controlled through the procedure administrative requirement for agreement of replicates *(*Type B Evaluation *).*
Temperature – all calibrators, control samples, and the measurand are brought to room temperature Variation in the time allowed to reach room temperature Variation in room temperature at different times of the year	Partially quantified in Type A Evaluation of process reproducibility data - blood matrix QC sample and partially through the procedure administrative requirement for agreement of replicates *(*Type B Evaluation*).*

532

Pipette diluter: Volume of sample, volume of internal standard, and dilution Calibration uncertainty or laboratory specification to verify calibration status	Type B Evaluation
Pipette diluter: Variation in use by multiple personnel	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Headspace vials: Crimping Material of stopper	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Time between replicate sampling of test item	Controlled through the procedure administrative requirement for agreement of replicates *(*Type B Evaluation*).*
Analysis	
Instrument parameter settings (e.g., oven temperature(s), gas flow, split ratios, aging of chromatographic column, autosampler syringe, autosampler precision, headspace equilibration time, headspace equilibration temperature, etc.)	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Interference from the matrix	Duplicate listing of component – see Calibrators section above.
Interference from reagents	This component is not an uncertainty component but is a quality control concern. The laboratory analyzes a matrix blank that contains no analyte but does evaluate all reagents used in the analytical method. The laboratory procedure specifies acceptable criteria for this control sample.
Interference from other compounds	Initially evaluated during method validation and determined to be insignificant, therefore not included in the uncertainty evaluation.
Stability of sample(s) from preparation through analysis	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample) and through the procedure administrative requirement for agreement of replicates (Type B Evaluation).
Instrument precision	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Systematic instrumental variation within an analytical batch	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample) and partially through the procedure administrative requirement for agreement of replicates *(*Type B Evaluation*).*
Data Processing	
Calibration model	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample and CRMs used as QC).
Integration parameters	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).
Processing algorithms	Adequately represented by the Type A Evaluation of process reproducibility data (Blood Matrix QC Sample).

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Type A Evaluation of uncertainty components

Measurement Process Reproducibility—Blood Matrix control sample

The number of observations of the blood matrix QC sample in this example exceeds 100. The statistic that will be calculated is the percent relative standard deviation.

To begin, the mean (average) and standard deviation of the blood matrix QC sample values will be calculated.[j]

The mean is calculated as:

$$\bar{x}=\frac{1}{n}\sum_{i=1}^{n} x_i=\frac{(x_1+x_2+x_3+\cdots+x_n)}{n}$$

The mean of the reproducibility data in this example is 0.0798 g/dL.

The standard deviation is calculated as:

$$s=\sqrt{\frac{\sum_{i=1}^{n}\left(x_i-\bar{x}\right)^2}{n-1}}$$

The standard deviation of the reproducibility data in this example is 0.0027 g/dL

Relative Standard Deviation (RSD) is calculated as:

$$RSD=\frac{s}{\bar{x}}$$

$$\%\ RSD=RSD\times 100\ \%$$

The %RSD of the reproducibility data in this example is:

$$RSD=\frac{0.0027\ g/dL}{0.0798\ g/dL}=0.0338$$

$$\%\ RSD=0.0338\times 100=3.38\ \%$$

Type B Evaluation of uncertainty components

Interference from the matrix

The laboratory evaluated matrix effects during method validation, which resulted in the test method incorporating a dilution factor using the pipette diluter. Dilution of the sample, in combination with

[j] For the readability of the example, the display of digits used in all calculations was abbreviated. Best practice is to include and carry all digits through all calculations and only round the reported value and its uncertainty to the proper number of significant figures.

the procedural requirements to mix the test item, minimizes matrix effects. The laboratory acknowledges that it is impossible to evaluate all variations in the test item matrix during method validation; therefore, the test method does include a blood matrix QC sample and a requirement for agreement between replicate samples to quantify the impact of the matrix on the measurement.

NOTE: The laboratory procedural requirement for replicate agreement is an example of an administrative control that restricts variation in the measurement method. It is up to a laboratory to determine if such an administrative control will be used. The decision may be based on, but not limited to, knowledge of the measurement process, the impact of repeat analysis on cost and process efficiency, and the required expanded uncertainty. Measurement data may sometimes exceed the administrative limit but may not be considered a statistical outlier, depending on its magnitude.

The laboratory procedure requires that two aliquots be taken from the homogenized test item. The measured ethanol concentrations of the two aliquots must be within ±5 % of the average, or the analysis is repeated.

The two uncertainty components – process reproducibility and interference from the matrix – quantify several of the same uncertainty components. The matrix control, over a longer period of time, holds the impact from the matrix constant while the effects from equipment, calibration, operators, and laboratory environmental conditions vary. The replicate samples of the test item provide information on the test item matrix and a short–term evaluation of the effect from equipment, calibration, operators, and the laboratory environment.

Calibrators: Uncertainty in the reference value

The laboratory reviewed the calibration certificates from all CRMs used for the calibration curve. The greatest uncertainty is 0.000233 g/dL for the 0.010 g/dL CRM.

$$\text{Relative uncertainty} = \left(\frac{0.000233\ \text{g/dL}}{0.010\ \text{g/dL}}\right) * 100 = 2.33\ \%$$

Pipette Diluter

The laboratory has set internal criteria for combined aliquots from both syringes: ±3 % for the internal standard syringe and ±3 % for the sample syringe. This helps ensure the proper functioning of the pipette diluter. It is noted that ±3 % is greater than the specifications for calibration used by the external calibration laboratory. Additionally, the procedure to ensure proper functioning is performed quarterly compared to the external calibration, which is performed annually. Therefore, the laboratory criteria of ±3 % for each syringe will be used to quantify variability for this uncertainty component.

Step 4—Convert quantities to standard uncertainties

The measurement unit

In this example, the relative uncertainty is expressed as a percentage.

Type A Evaluation of uncertainty components

Measurement Process Reproducibility Data

Test specimens are sampled in duplicate, analyzed in two separate batches and the laboratory procedure for the reported ethanol concentration is to average the two results. Therefore, the %RSD of the mean is calculated by taking the %RSD of the measurement process and dividing by the square root of the number of measurements averaged to generate the reported ethanol concentration.

The %RSD of the reproducibility data in this example is 3.38 %

The mathematical expression for %RSD of the mean:

$$\%\text{RSD}_{\text{mean}} = \frac{\%\text{RSD}}{\sqrt{n}}$$

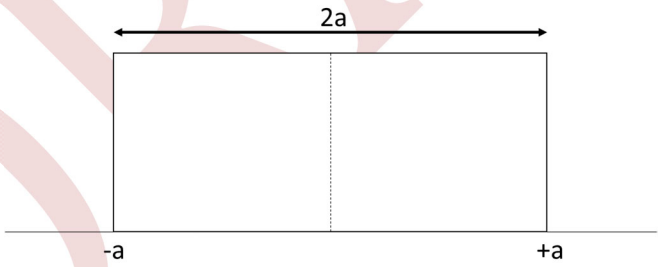
The %RSD of the mean of the reproducibility data in this example is:

$$\%\text{RSD}_{\text{mean}} = \frac{3.38\ \%}{\sqrt{2}} = 2.3900\ \%$$

Type B Evaluation of uncertainty components

Homogenization

The laboratory procedure requires two samples to be taken from the homogenized test specimens and the ethanol concentration of the two aliquots to be within ±5 % of the average, or the analysis is repeated. This component is evaluated as a rectangular distribution:



Upper limit = +a

Lower limit = -a

Possible range of values = (+a) – (-a) = 2a

For a rectangular distribution, the standard uncertainty is calculated by:

$$\text{Standard uncertainty} = \frac{a}{\sqrt{3}}$$

The standard uncertainty for the interference from the matrix in this example is based on an outside limit of 5 %:

$$\text{Standard uncertainty} = \frac{5\ \%}{\sqrt{3}} = 2.8868\ \%$$

Calibrators: Uncertainty in the reference value

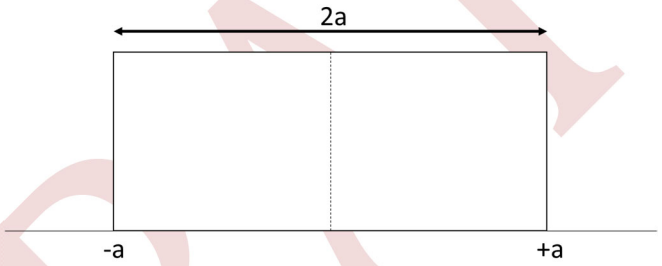
Based on the certificates from the CRMs used for calibrators in this method, the laboratory determined in Step 3 that the greatest relative uncertainty for the CRMs is 2.33 %.

The certificate indicates that this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2, and a coverage probability of approximately 95 %. The uncertainty on the calibration certificate will be divided by the coverage factor to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty} = \left(\frac{2.33\ \%}{2}\right) = 1.1650\ \%$$

Pipette Diluter

In Step 3, the laboratory determined that its in-house criteria of ±3 % will be used to quantify variability for this uncertainty component for both the sample and internal standard syringes. This component is evaluated as a rectangular distribution:



Upper limit = +a

Lower limit = -a

Possible range of values = (+a) – (-a) = 2a

As explained above, for a rectangular distribution, the standard uncertainty is calculated by:

$$\text{Standard uncertainty} = \frac{a}{\sqrt{3}}$$

The standard uncertainty for the pipette diluter sample ($u_{\text{sample syringe}}$) and internal standard syringes ($u_{\text{IS syringe}}$) in this example is based on the outside limit of 3 %:

$$u_{\text{sample syringe}} = \frac{3\ \%}{\sqrt{3}} = 1.7321\ \%$$

$$u_{\text{IS syringe}} = \frac{3\ \%}{\sqrt{3}} = 1.7321\ \%$$

Step 5—Calculate the combined standard uncertainty

The evaluation will assume that the uncertainty components are independent or uncorrelated and that the measurement result is the sum of a series of components.

Care shall be taken if the measurement results lie over a range of values. In this scenario, the calibration model was determined during method validation and shown through residual plots to have constant variance across the linear range, so a single measurement uncertainty can be calculated for the entire concentration range.

$$u_c(y)=\sqrt{s^2_{\text{reproducibility}}+u^2_{\text{homogenization}}+u^2_{\text{CRMunc}}+u^2_{\text{sample syringe}}+u^2_{\text{IS syringe}}}$$

$$u_c(y)=\sqrt{2.3900^2_{\text{reproducibility}}+2.8868^2_{\text{homogenization}}+1.1650^2_{\text{CRMunc}}+1.7321^2_{\text{sample syringe}}+1.7321^2_{\text{IS syringe}}}$$

$$u_c(y)=\sqrt{21.4033}$$

$$u_c(y)=4.6264\%$$

Evaluation of bias

The laboratory views bias monitoring as a component of ensuring the validity of the test method. It has incorporated three CRMs at low, medium, and high concentrations as QC samples to monitor bias from unidentified sources on an ongoing basis.

The laboratory procedure requires each measured value for a CRM to be within 5 % of the reference value. The largest bias for any of the control levels (low, medium, and high) is less than the combined standard uncertainty. Although the bias is viewed as insignificant, the laboratory is choosing to include an additional component in the uncertainty evaluation that will address the uncertainty in the reference value of the CRM used to evaluate bias. Steps 3, 4, and 5 must be addressed for this additional uncertainty component.

Step 3—Quantify uncertainty components – bias component

The laboratory reviewed all of the certificates of analysis from all CRMs used for the evaluation of bias. The greatest uncertainty is 0.0014 g/dL for the 0.3 g/dL CRM.

$$\text{Relative uncertainty}=\left(\frac{0.0014\ \text{g/dL}}{0.3\ \text{g/dL}}\right)*100=0.4667\ \%$$

Step 4—Convert quantities to standard uncertainties – bias component

The certificate indicates this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2, and a coverage probability of approximately 95 %. The uncertainty on the calibration certificate will be divided by the coverage factor, 2, to arrive at a standard uncertainty.

$$\text{Relative standard uncertainty} = \left(\frac{0.4667\ \%}{2}\right) = 0.2334\ \%$$

Step 5—Calculate combined standard uncertainty – including bias component

The revised RSS calculation:

$$u_c(y) = \sqrt{s^2_{\text{reproducibility}} + u^2_{\text{homogenization}} + u^2_{\text{CRMunc}} + u^2_{\text{sample syringe}} + u^2_{\text{IS syringe}} + u^2_{\text{CRMbias}}}$$

$$u_c(y) = \sqrt{2.3900^2_{\text{reproducibility}} + 2.8868^2_{\text{homogenization}} + 1.1650^2_{\text{CRMunc}} + 1.7321^2_{\text{sample syringe}} + 1.7321^2_{\text{IS syringe}} + 0.2334^2_{\text{CRMbias}}}$$

$$u_c(y) = \sqrt{21.4578}$$

$$u_c(y) = 4.6323\ \%$$

Step 6—Expand the combined standard uncertainty by coverage factor (*k*)

The data from the measurement process has demonstrated that the measurement results follow a normal distribution. The laboratory has 101 measurements of the blood matrix control sample. Therefore, the laboratory assumes a lower bound on the effective degrees of freedom (n-1) for the combined standard uncertainty of 100.

To expand the uncertainty to a 95.45 % coverage probability for this example, the coverage factor k = 2.025 from the Student's t-distribution table for 100 degrees of freedom will be used.

$$U = 2.025 \times 4.6323 = 9.3804\ \%$$

NOTE: A laboratory can choose to increase the coverage probability.

Step 7—Evaluate the expanded uncertainty

The laboratory determined that the evaluation of uncertainty is fit for purpose based on the following considerations:

— Stakeholder interests

Expanded uncertainty (9.3804 %) was below a stakeholder specification of 10 %.

— Legal requirements

There were none.

694 — The relationship between the reported test value and the expanded MU

695 Expanded uncertainty as a percentage across the analytical range ensures a consistent
696 relationship.

697 — Established criteria, including control limits for the method

698 The laboratory's control acceptance limits for the method are 10 %. Considering the expanded
699 uncertainty, the allowable control limits were determined to be acceptable.

700 **Step 8—Report the uncertainty**

701 The laboratory has established a procedure for rounding the expanded uncertainty. Following that
702 procedure, the expanded uncertainty was rounded to two significant figures:

703 U=9.4 %

704 For reporting measurement results with the rounded expanded uncertainty to the same number of
705 decimal places:

706 *"The concentration of ethanol in Item 1 was found to be 0.090 g/dL ± 0.008 g/dL at a coverage*
707 *probability of 95.45 %."*

708

Uncertainty Budget Form					
Method:	The Concentration of Ethanol in Ante-Mortem Blood Using SOP #200				
Prepared By:	J. Smith		**Date:**	25-May-2023	
Sources of Uncertainty	**Type A or B?**	**Std Dev or Outside Limits**	**Distribution Model**	**Divisor**	**Std Uncertainty (1σ)**
Measurement Process Reproducibility ($s_{reproducibility}$)	A	3.38 %	Normal	√2	2.3900 %
Homogenization / Matrix Interference (u_{matrix})	B	5.00 %	Rectangular	√3	2.8868 %
Calibrators: Uncertainty in Ref Value (u_{CRMunc})	B	2.33 %	Normal	2	1.1650 %
Pipette Diluter – Sample Syringe ($u_{sample\ syringe}$)	B	3.00 %	Rectangular	√3	1.7321 %
Pipette Diluter – Internal Standard Syringe ($u_{IS\ syringe}$)	B	3.00 %	Rectangular	√3	1.7321 %
Bias Component ($u_{CRMbias}$)	B	0.4667 %	Normal	2	0.2334 %
Combined Uncertainty ($u_c(y)$):	4.6323 %				
Confidence Level (k):	95.45 % (k = 2.025)				
Expanded Uncertainty (U):	9.3804 % **(9.4 %)**				

709 **Figure A.1: Uncertainty Budget Form-Ethanol in Antemortem Blood Using SOP #200** [j]

Annex B
(informative)

Concentration of Amphetamine and Methamphetamine in a Whole Blood Specimen[k]

Test Method Information

The laboratory developed and validated a test method for quantitating amphetamine and methamphetamine in whole blood using liquid chromatography-tandem mass spectrometry (LC-MSMS). Multiple personnel were trained and qualified to use the laboratory's procedure. All personnel use the same equipment for this test method. Analytical results are normalized to internal standards added during the sample preparation process.

The method is calibrated using single replicates of whole blood fortified calibrators at 6 concentrations from 10 to 1000 ng/mL. The calibrators are prepared from a working stock solution made by diluting certified reference materials (CRMs). The working stock solution is fortified into whole blood with each batch. Method validation determined that the proper calibration model was a quadratic regression model. A lack of consistent variance across the concentration range (heteroscedasticity) was observed.

The measurement results from single aliquots of a test specimen are reported.

Calibrators, QC samples, and test specimens are aliquoted at the same time using the same equipment.

Metrological Traceability

The traceability of this measurement process is established through the calibrators used to generate the calibration curve on the measuring system and through the calibration of other equipment used in the measurement process.

All CRMs have been purchased from a Reference Material Producer that meets the ANSI/ASB Standard 017, *Standard for Metrological Traceability in Forensic Toxicology*.

All external calibrations of measuring equipment (e.g., volumetric flasks and pipettes) are performed by calibration laboratories that meet the *ANSI/ASB Standard 017, Standard for Metrological Traceability in Forensic Toxicology*.

Measurement Assurance

The QC samples at low (30 ng/mL), medium (400 ng/mL), and high (800 ng/mL) concentrations are fortified into whole blood from a working stock solution by the laboratory with each batch. The working stock solution for the controls is prepared from CRMs purchased from a different supplier

[k] An evaluation of measurement uncertainty is specific to the metrological traceability established for the measurement, the measurement assurance processes in place, the laboratory test method, the laboratory facility, etc. Therefore, the following is only an example for evaluation and revision by a laboratory after considering the elements specific to that laboratory.

than the CRMs used as calibrators. The QC samples are used to ensure the validity of the test method across the concentration range and to evaluate the method's bias on an ongoing basis.

The laboratory has 15 measurements made of the QC samples during validation for each concentration.

Two separate uncertainty evaluations will be needed since two analytes are involved in this measurement procedure.

Step 1—Specify the measurement process

The measurement processes can be described in a written statement:

"The Concentration of Amphetamine in Whole Blood using [the validated laboratory procedure]"

"The Concentration of Methamphetamine in Whole Blood using [the validated laboratory procedure]"

Step 2—Identify uncertainty components

The following list of possible contributors to uncertainty in this method was identified by the laboratory:

<u>Personnel</u>

- Inter-personnel variation in sample preparation and measurements
- Training
- Experience

<u>Calibrators Preparation</u>

- Components:
  - Methanol – reagent grade
  - Concentration – equipment used to prepare (pipettes, volumetric flask)
- CRMs – uncertainty in the stated reference value

<u>Control Preparation</u>

- Components:
  - Methanol – reagent grade
  - Concentration – equipment used to prepare (pipettes, volumetric flask)
- CRMs – uncertainty in the stated reference value

Internal Standard Preparation

- Components:
    - Methanol – reagent grade
    - Stable isotope labeled amphetamine and methamphetamine
- Impurities in the internal standard (unlabeled drug)
- Concentration – equipment used to prepare (pipettes, volumetric flask)

Preparation of aliquots of Calibrators, Control Samples, and Measurand

- Homogenization
    - Test Specimens – mixing
- Temperature
    - All calibrators, control samples, and test specimens are brought to room temperature
        - Variation in the time allowed to reach room temperature
        - Variation in room temperature at different times of year
- Pipettes
    - Volume of sample, calibrators, controls, and internal standard
        - Calibration uncertainty or laboratory specification to verify calibration status

Analysis

- Instrument parameter settings (e.g., gradient, flow rate, aging of the chromatographic column, autosampler syringe, autosampler precision)
- Interference from the matrix
- Interference from reagents
- Interference from other compounds
- Stability of sample(s) from preparation through analysis
- Instrument precision
- Systematic instrumental variation within an analytical batch
- Matrix effect (ionization suppression/enhancement)

Data Processing

— Calibration model

— Integration parameters

— Processing algorithms

NOTE 1: This list of uncertainty components could also be compiled into a fishbone diagram or any other format of the laboratory's choosing.

NOTE 2: A laboratory may identify different uncertainty components when evaluating its specific measurement process.

Step 3—Quantify uncertainty components

The laboratory has validation data from the measurement process:

— The calibration model was determined during method validation and was shown using a statistical test to have some heteroscedasticity (the variance was not constant across the linear range). Therefore, the laboratory will evaluate the measurement uncertainty using data from the control with the largest variance and apply it to the entire reportable concentration range.

— The QC samples at low (30 ng/mL), medium (400 ng/mL), and high (800 ng/mL) concentrations are fortified into whole blood from a working stock solution by the laboratory with each batch. All personnel have contributed to the 15 replicate measurements of the control samples at each concentration.

Table B.1 shows the individual uncertainty components and how they will be evaluated.

816 **Table B.1—Method of Evaluation of Uncertainty Components**

Uncertainty Component	Method of Evaluation
Personnel	
Inter-personnel variation	Adequately represented by the Type A Evaluation of process reproducibility data
Training	Adequately represented by the Type A Evaluation of process reproducibility data
Experience	Adequately represented by the Type A Evaluation of process reproducibility data
Calibrators Preparation	
Components: Methanol – reagent grade	Adequately represented by the Type A Evaluation of process reproducibility data
Concentration CRM – uncertainty in the stated reference value Equipment used to prepare (pipettes, volumetric flask)	Type B Evaluation
Control Samples Preparation	
Components: Methanol – reagent grade	Adequately represented by the Type A Evaluation of process reproducibility data
Concentration CRM – uncertainty in the stated reference value Equipment used to prepare (pipettes, volumetric flask)	Type B Evaluation *(if necessary for bias)*
Internal Standard Preparation	
Components: Methanol – reagent grade	Adequately represented by the Type A Evaluation of process reproducibility data
Stable isotope labeled amphetamine and methamphetamine Impurities in the internal standard (unlabeled drug)	No influence A certificate of analysis from the reference material provider indicates no impurity The measurement result will only be impacted by the volume of the internal standard added to each sample
Concentration- equipment used to prepare (pipettes, volumetric flask)	No influence Procedural requirement to use the same lot of Internal Standard for all samples in an analytical batch

817

Preparation of aliquots of Calibrators, Control Samples, and Test Specimens	
Homogenization – mixing	Demonstrated during method validation to be insignificant.
Temperature – all calibrators, controls, and the measurand are brought to room temperature Variation in the time allowed to reach room temperature Variation in room temperature at different times of year	Adequately represented by the Type A Evaluation of process reproducibility data
Pipettes: Volume of sample, calibrators, controls, and internal standard Calibration uncertainty or laboratory specification to verify calibration status	Volume of internal standard adequately represented by the Type A Evaluation of process reproducibility data Type B Evaluation for volume of sample and calibrators (for controls only if necessary for bias)
Analysis	
Instrument parameter settings (e.g., gradient, flow rate, aging of chromatographic column, autosampler syringe, autosampler precision, etc.)	Adequately represented by the Type A Evaluation of process reproducibility data
Interference from the matrix	Matrix interference was evaluated during method validation and found insignificant for the matrix type allowed in this method.
Interference from reagents	This component is not an uncertainty component but a quality control concern. The laboratory analyzes a matrix blank that contains no analyte and evaluates all reagents used in the analytical method. The laboratory procedure specifies acceptable criteria for this control sample.
Interference from other compounds	Demonstrated lack of interference from other compounds during method validation. This component is not considered an uncertainty component.
Stability of sample(s) from preparation through analysis	Adequately represented by the Type A Evaluation of process reproducibility data
Instrument precision	Adequately represented by the Type A Evaluation of process reproducibility data
Systematic instrumental variation within an analytical batch	The positive controls are reinjected at the end of the batch and must meet predefined criteria
Data Processing	
Calibration model	Adequately represented by the Type A Evaluation of process reproducibility data
Integration parameters	Adequately represented by the Type A Evaluation of process reproducibility data
Processing algorithms	Adequately represented by the Type A Evaluation of process reproducibility data

Type A Evaluation of uncertainty components

Measurement Process Reproducibility

Each QC sample has 15 observations. The statistic that will be calculated is the percent relative standard deviation.

During validation, control data demonstrated a lack of consistent variance across the calibration range. Therefore, the reproducibility data from the multiple QC sample levels for either target compound may not be combined. The 400 ng/mL QC sample had the greatest variance and will be used for this evaluation.

To begin, the control data's mean (average) and standard deviation will be calculated.

- The mean of the reproducibility data in this example is 404 ng/mL for amphetamine and 416 ng/mL for methamphetamine.

- The standard deviation of the reproducibility data in this example is 15.90 ng/mL for amphetamine and 12.01 ng/mL for methamphetamine.

The %RSD of the reproducibility data in this example is 3.9356 % for amphetamine and 2.8870 % for methamphetamine.

Type B Evaluation of uncertainty components

Calibrators Preparation

Uncertainty in the reference value

The laboratory reviewed the calibration certificates from all CRMs used for the preparation of the calibration working stock solutions. The largest uncertainty was 0.005 mg/mL for the 1.000 mg/mL amphetamine CRM and 0.006 mg/mL for the 1.000 mg/mL methamphetamine CRM.

$$\text{Relative uncertainty of Amphetamine CRM} = \left(\frac{0.005\ \text{mg/mL}}{1.000\ \text{mg/mL}}\right) * 100 = 0.5\ \%$$

$$\text{Relative uncertainty of Methamphetamine CRM} = \left(\frac{0.006\ \text{mg/mL}}{1.000\ \text{mg/mL}}\right) * 100 = 0.6\ \%$$

Uncertainty in pipettes

The laboratory reviewed the calibration certificates of all pipettes that may be used for preparation of the calibration working stock solution. The largest uncertainty was 0.74 µL for a 100µL pipette.

$$\text{Relative uncertainty of Pipettes to Prep Cal Working Stock} = \left(\frac{0.74\ \mu\text{L}}{100\ \mu\text{L}}\right) * 100 = 0.74\ \%$$

847 ***Uncertainty in volumetric flasks***

848 The laboratory reviewed the calibration certificates of all volumetric flasks that may be used for
849 preparation of the calibration working stock solution. The largest uncertainty was 0.0086 mL for a
850 25mL volumetric flask.

851 $$\text{Relative uncertainty of Vol Flask to Prep Cal Working Stock} = \left(\frac{0.0086\ \text{mL}}{25\ \text{mL}}\right) * 100 = 0.0344\ \%$$

852 **Preparation of aliquots of Calibrators and Test Specimens**

853 ***Uncertainty in pipettes***

854 The laboratory reviewed the calibration certificates of all pipettes that may be used to fortify the
855 calibrators from the working stock solution into whole blood. The method requires the same pipette
856 to be used to add the internal standard to calibrators, controls, and test specimens. The largest
857 uncertainty was 0.74 µL for a 100-µL pipette.

858 $$\text{Relative uncertainty of Pipettes to Fortify Calibrator Samples} = \left(\frac{0.74\ \mu\text{L}}{100\ \mu\text{L}}\right) * 100 = 0.74\ \%$$

859 $$\text{Relative uncertainty of Pipettes to Delivery Internal Standard} = \left(\frac{0.74\ \mu\text{L}}{100\ \mu\text{L}}\right) * 100 = 0.74\ \%$$

860 The laboratory reviewed the calibration certificates of all pipettes that may be used to aliquot the test
861 item. The largest uncertainty was 6.9 µL for a 1000-µL pipette.

862 $$\text{Relative uncertainty of Pipettes to Aliquot Test Samples} = \left(\frac{6.9\ \mu\text{L}}{1000\ \mu\text{L}}\right) * 100 = 0.69\ \%$$

863 **Step 4—Convert quantities to standard uncertainties**

864 **The measurement unit**

865 In this example, the relative uncertainty is expressed as a percentage.

866 **Type A Evaluation of uncertainty components**

867 **Measurement Process Reproducibility Data**

868 The % RSD (s_r) of the reproducibility data in this example is 3.9356 % for amphetamine and 2.8870
869 % for methamphetamine.

870

Type B Evaluation of uncertainty components

Calibrators Preparation

Uncertainty in the reference value

Based on the certificates from the CRMs used to prepare the calibrator working stock solutions in this method, the laboratory determined in Step 3 that the relative uncertainty is 0.5 % and 0.6 % for amphetamine and methamphetamine, respectively.

The certificates indicate that the expanded uncertainties assume a normal distribution, a coverage factor of k = 2, and a coverage probability of approximately 95 %. The relative uncertainties will be divided by the coverage factor to arrive at relative standard uncertainties.

$$\text{Relative standard uncertainty of Amphetamine CRM} = \left(\frac{0.5\ \%}{2}\right) = 0.2500\ \% = u_{CRM}$$

$$\text{Relative standard uncertainty of Methamphetamine CRM} = \left(\frac{0.6\ \%}{2}\right) = 0.300\ \% = u_{CRM}$$

Uncertainty in pipettes

In Step 3, the laboratory determined that among the pipettes used to prepare the working stock solutions, the largest relative uncertainty was 0.74 % for a 100-µL pipette.

The pipette's calibration certificate indicates that this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2.87, and a coverage probability of approximately 95 %. The relative uncertainty derived from the calibration certificate will be divided by the coverage factor, 2.87, to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty of Pipettes to Prep Calib Working Stock} = \left(\frac{0.74\ \%}{2.87}\right) = 0.2578\ \% = u_{CRMp}$$

Uncertainty in volumetric flasks

In Step 3, the laboratory determined that among the volumetric flasks used to prepare the working stock solutions, the largest relative uncertainty was 0.0344 % for a 25-mL flask.

The volumetric flask's calibration certificate indicates that this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2, and a coverage probability of approximately 95 %. The relative uncertainty derived from the calibration certificate will be divided by the coverage factor, 2, to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty of Vol Flasks to Prep Calib Working Stock} = \left(\frac{0.0344\ \%}{2}\right) = 0.0172\ \% = u_{CRMv}$$

Preparation of aliquots of Calibrators and Test Specimens

Uncertainty in pipettes

In Step 3, the laboratory determined that among the pipettes used to fortify the calibrators from the working stock solution into whole blood, the largest relative uncertainty was 0.74 % for a 100µL pipette. The same pipette is used to fortify all samples with the internal standards.

The pipette's calibration certificate indicates this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2.87, and a coverage probability of approximately 95 %. The uncertainty derived from the calibration certificate will be divided by the coverage factor to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty of Pipettes to Fortify Calibrator Samples} = \left(\frac{0.74\ \%}{2.87}\right) = 0.2578\ \% = u_{CALp}$$

$$\text{Relative standard uncertainty of Pipette to Deliver Internal Standard} = \left(\frac{0.74\ \%}{2.87}\right) = 0.2578\ \% = u_{ISp}$$

In Step 3, the laboratory also determined that among the pipettes used to aliquot test specimens, the largest relative uncertainty was 0.69 % for a 1000-µL pipette.

The pipette's calibration certificate indicates that this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2.87, and a coverage probability of approximately 95 %. The uncertainty on the calibration certificate will be divided by the coverage factor, 2.87, to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty of Pipettes to Aliquot Test Samples} = \left(\frac{0.69\ \%}{2.87}\right) = 0.2404\ \% = u_{ITEMp}$$

Step 5—Calculate the combined standard uncertainty

The evaluation will assume that the uncertainty components are independent or uncorrelated and that the measurement result is the sum of a series of components.

For Amphetamine:

$$u_c(y) = \sqrt{3.9356_r^2 + 0.2500_{CRM}^2 + 0.2578_{CRMp}^2 + 0.0172_{CRMv}^2 + 0.2578_{CALp}^2 + 0.2578_{ISp}^2 + 0.2404_{ITEMp}^2}$$

$$u_c(y) = \sqrt{15.8089}$$

$$u_c(y) = 3.9760\ \%$$

For Methamphetamine:

$$u_c(y) = \sqrt{2.8870_r^2 + 0.3000_{CRM}^2 + 0.2578_{CRMp}^2 + 0.0172_{CRMv}^2 + 0.2578_{CALp}^2 + 0.2578_{ISp}^2 + 0.2404_{ITEMp}^2}$$

$$u_c(y) = \sqrt{8.6822}$$

$$u_c(y) = 2.9466\ \%$$

Evaluation of bias

In this example, the laboratory views bias monitoring as a component of ensuring the validity of the test method and has incorporated three controls prepared from CRMs at low, medium, and high concentrations as QC samples to monitor bias from unidentified sources on an ongoing basis.

The largest average bias for any of the control levels (low, medium, and high) during validation was –2.4 % for amphetamine and 4.0 % for methamphetamine.

The bias for amphetamine is less than the combined standard uncertainty (3.9765 %) and is, therefore, insignificant. No additional component for bias will be added.

The bias for methamphetamine is greater than the combined standard uncertainty (2.9476 %) and is, therefore, significant. For the methamphetamine bias component, Steps 3, 4, and 5 must be addressed.

Step 3—Quantify uncertainty components – bias component

During validation, the largest bias for methamphetamine was quantified to be 4.0 %.

Step 4—Convert quantities to standard uncertainties – bias component

The laboratory has chosen the option explained in Section 5.6.2.4 c) to address the bias for methamphetamine that was determined to be significant. Following the guidance in Section 3.2.5.5 of NIST SOP 29, the bias is treated as an uncorrected systematic error, and the following equation applying a rectangular distribution is used to address the uncertainty of the difference component (u_d) in the MU evaluation:

$$u_d = \frac{\text{bias}}{\sqrt{3}} = \frac{4.0}{\sqrt{3}} = 2.3094$$

Step 5—Calculate combined standard uncertainty – including bias component

For Methamphetamine, the updated root sum of the squares:

$$u_c(y) = \sqrt{2.8870_r^2 + 0.3000_{CRM}^2 + 0.2578_{CRMp}^2 + 0.0172_{CRMv}^2 + 0.2578_{CALp}^2 + 0.2578_{ISp}^2 + 0.2404_{ITEMp}^2 + 2.3094_d^2}$$

$$u_c(y) = \sqrt{14.0156}$$

$$u_c(y) = 3.7437\ \%$$

Step 6—Expand the combined standard uncertainty by coverage factor (*k*)

The data from the measurement process is assumed to follow a normal distribution. The laboratory has 15 measurements of the 400 ng/mL QC control. Therefore, the laboratory assumes that the effective degrees of freedom (*n*-1) for the combined standard uncertainty cannot be lower than 14.

Refer to the Student's *t*-distribution table to determine the *k* factor for 14 degrees of freedom.

For this example, the coverage factor k = 2.20 will expand the uncertainty to a 95.45 % coverage probability.

For Amphetamine:

$$U=2.20\times3.9760=8.7472\ \%$$

For Methamphetamine:

$$U=2.20\times3.7437=8.2362\ \%$$

Step 7—Evaluate the expanded uncertainty

The laboratory determined that the evaluation of uncertainty is fit-for-purpose based on the following considerations:

— Stakeholder interests

There were none.

— Legal requirements

There were none.

— The relationship between the reported test value and the expanded MU

Expanded uncertainty as a percentage across the analytical range ensures a consistent relationship.

— Established criteria including control limits for method

The laboratory's control limits for the method are 20 %. The control limits were not revised as the MU was based only on validation data. The decision was made to review quality control data on a quarterly basis to evaluate whether control limits should be revised.

Step 8—Report the uncertainty

The laboratory has established a procedure for rounding the expanded uncertainty. Following that procedure, the expanded uncertainty rounded to two significant figures:

For Amphetamine:

U = 8.7 %

For Methamphetamine:

U = 8.2 %

For reporting measurement results with the rounded expanded uncertainties to the same number of decimal places:

"The concentration of amphetamine in Item 1 was found to be 90 ± 8 ng/mL at a coverage probability of 95.45 %. The concentration of methamphetamine in Item 1 was found to be 143 ± 12 ng/mL at a coverage probability of 95.45 %."

Uncertainty Budget Form					
Method:	The Concentration of Amphetamine in Whole Blood Using SOP AMPH-536				
Prepared By:	J. Smith		**Date:**	15-Jun-2023	
Sources of Uncertainty	**Type A or B?**	**Std Dev or Outside Limits**	**Distribution Model**	**Divisor**	**Std Uncertainty (1σ)**
Measurement Process Reproducibility (s_r)	A	3.9356 %	Normal	1	3.9356 %
Calibrators: Uncertainty in Reference Value (u_{CRM})	B	0.5 %	Normal	2	0.2500 %
Pipette – Prep Calibrator Working Stock (u_{CRMp})	B	0.74 %	Normal	2.87	0.2578 %
Vol Flask – Prep Calibrator Working Stock (u_{CRMv})	B	0.0344 %	Normal	2	0.0172 %
Pipette – Fortify Calibrator Samples (u_{CALp})	B	0.74 %	Normal	2.87	0.2578 %
Pipette – Deliver Internal Standard (u_{ISp})	B	0.74 %	Normal	2.87	0.2578 %
Pipette – Aliquot Test Samples (u_{ITEMp})	B	0.69 %	Normal	2.87	0.2404 %
Combined Uncertainty ($u_c(y)$):	3.9760 %				
Confidence Level (k):	95.45 % (k = 2.20)				
Expanded Uncertainty (U):	8.7472 % **(8.7 %)**				

Figure B.1: Uncertainty Budget Form-Amphetamine in Whole Blood Using SOP AMPH-536 [j]

993

Uncertainty Budget Form					
Method:	The Concentration of Methamphetamine in Whole Blood Using SOP AMPH-536				
Prepared By:	J. Smith		**Date:**	15-Jun-2023	
Sources of Uncertainty	**Type A or B?**	**Std Dev or Outside Limits**	**Distribution Model**	**Divisor**	**Std Uncertainty (1σ)**
Measurement Process Reproducibility (s_r)	A	2.8870 %	Normal	1	2.8870 %
Calibrators: Uncertainty in Reference Value (u_{CRM})	B	0.6 %	Normal	2	0.3000 %
Pipette – Prep Calibrator Working Stock (u_{CRMp})	B	0.74 %	Normal	2.87	0.2578 %
Vol Flask – Prep Calibrator Working Stock (u_{CRMv})	B	0.0344 %	Normal	2	0.0172 %
Pipette – Fortify Calibrator Samples (u_{CALp})	B	0.74 %	Normal	2.87	0.2578 %
Pipette – Deliver Internal Standard (u_{ISp})	B	0.74 %	Normal	2.87	0.2578 %
Pipette – Aliquot Test Samples (u_{ITEMp})	B	0.69 %	Normal	2.87	0.2404 %
Bias Component (u_d)	B	4.0 %	Rectangular	√3	2.3094 %
Combined Uncertainty ($u_c(y)$):	3.7437 %				
Confidence Level (k):	95.45 % (k = 2.20)				
Expanded Uncertainty (U):	8.2362 % **(8.2 %)**				

994 **Figure B.2: Uncertainty Budget Form-Methamphetamine in Whole Blood Using SOP AMPH-536**

995

Annex C
(informative)

Calibration of Breath Alcohol Measuring Instrumentation Using Long-Term Calibration Data from a Single Instrument[l]

Calibration Method Information

The calibration of an individual breath alcohol instrument uses dry gas measurement standard data from the current calibration as well as historical calibration data for this single instrument over time. The calibration method uses measurement standards at multiple concentrations ranging from 0.040 g/210 L to 0.300 g/210 L.

The calibration method does require each concentration of the dry gas measurement standards to be evaluated in triplicate. The method requires each triplicate measurement to be within 3 % or 0.003 g of ethanol/210 L of breath (g/210 L), whichever is greater, of the certified reference value of the measurement standard. Furthermore, the method requires that there shall be no greater than 0.003 g/210 L difference in all three measurements at each concentration.

Step 1—Specify the measurement process

Calibration of breath alcohol measuring instrumentation using long-term calibration data from a single instrument

Step 2—Identify uncertainty components

The following list of possible contributors to uncertainty in the calibration method were identified:

Personnel

— Inter-personnel variation in performing calibration

— Training

— Experience

Breath Alcohol Measuring Instrument Being Calibrated

— Variability of instrument over time

Measurement Standards

— Dry Gas Certified Reference Materials - uncertainty in the stated reference value

[l] An evaluation of measurement uncertainty is specific to the metrological traceability established for the measurement, the measurement assurance processes in place, the breath alcohol program calibration method, the laboratory facility, etc. Therefore, the following is only an example for evaluation and revision by a breath alcohol program after considering the elements specific to that program.

Environmental Conditions

— Barometric pressure

— Humidity

— Temperature

Varying Facilities/Location Change

— Instrument transport

— Power fluctuation

Data Processing

— Processing algorithms

Step 3—Quantify uncertainty components

Measurement standard data has been collected from use of this calibration method over time. All personnel have participated in acquiring the measurement standard data for this single breath alcohol measuring instrument. The laboratory has 51 measurements made using each measurement standard. The instrument has not demonstrated consistent variance across the concentration range of the measurement standards used in the calibration method. Because the 0.100 g/210 L measurement standard has the greatest observed variance of the measurement standards, it will be used to represent the process reproducibility data.

Table C.1 shows the individual uncertainty components and how they will be evaluated.

Table C.1—Method of Evaluation of Uncertainty Components

Uncertainty Component	Method of Evaluation
Personnel	
Inter-personnel variation	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Training	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Experience	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Breath Alcohol Measuring Instrument Being Calibrated	
Variability of the instrument over time	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Measurement Standards	
CRM –uncertainty in the stated reference value	Type B Evaluation
Environmental Conditions	
Barometric pressure	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Humidity	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Temperature	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard
Varying Facilities/Locations	
Instrument transport	Not Applicable
Power fluctuations	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard.
Data Processing	
Processing algorithms	Adequately represented by Type A Evaluation of process reproducibility data – measurement standard

Type A Evaluation of uncertainty components

Measurement Standard Reproducibility – 0.100 g/210 L Measurement Standard

The number of observations in this example is 51. The statistic that will be calculated is the standard deviation.

To begin, the measurement data's mean (average) and standard deviation will be calculated.[m]

[m] For the readability of the example, the display of digits used in all calculations was abbreviated. Best practice is to include and carry all digits through all calculations and only round the reported value and its uncertainty to the proper number of significant figures.

The mean is calculated as:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

$$\bar{x} = \frac{(x_1 + x_2 + x_3 + \ldots x_n)}{n}$$

The mean of the reproducibility data in this example = 0.0994 g/210 L

The standard deviation is calculated as:

$$s = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{n-1}}$$

The standard deviation of the reproducibility data in this example = 0.0012 g/210 L

Type B Evaluation of uncertainty components

Certified Reference Materials

Based on the certificates from the CRMs, the laboratory determined in Step 3 that the greatest relative uncertainty for the CRM was 0.0018 g/210 L for the 0.100 g/210 L CRM.

The certificate indicates this expanded uncertainty assumes a normal distribution, a coverage factor of k = 2, and a coverage probability of approximately 95 %. The uncertainty on the calibration certificate will be divided by the coverage factor to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty} = \left(\frac{0.0018\ \text{g}/210\text{L}}{2}\right) = 0.0009\ \frac{\text{g}}{210}\text{L}$$

Step 4—Convert quantities to standard uncertainties

The measurement unit: g of ethanol/210 L of breath (g/210 L)

Type A Evaluation of uncertainty components

Measurement Standard Reproducibility – 0.100 g/210 L Measurement Standard

The standard deviation of the reproducibility data in this example is 0.0012 g/210 L.

— No additional conversion is necessary to reach a standard uncertainty.

Type B Evaluation of uncertainty components:

Certified Reference Materials

The CRM certificate indicates that the stated expanded uncertainty assumes a normal distribution, a coverage factor of $k = 2$, and a coverage probability of approximately 95 %.

— The uncertainty is stated to be 0.0018 g/210 L for the 0.100 g/210 L CRM.

— The uncertainty on the calibration certificate will be divided by the coverage factor, 2, to arrive at a standard uncertainty.

— 0.0018 g/210 L /2 = 0.0009 g/210 L for the standard uncertainty

Step 5—Calculate combined standard uncertainty

The evaluation will assume that the uncertainty components are independent or uncorrelated and that the measurement result is the sum of a series of components. The combined standard uncertainty was calculated.

$$u_c(y)=\sqrt{s^2_{\text{reproducibility}}+u^2_{\text{CRMunc}}}$$

$$u_c(y)=\sqrt{0.0012^2_{\text{reproducibility}}+0.0009^2_{\text{CRMunc}}}$$

$$u_c(y)=\sqrt{0.0012^2_{\text{reproducibility}}+0.0009^2_{\text{CRMunc}}}$$

$$u_c(y)=\sqrt{2.25\text{x}10^{-6}}$$

$$u_c(y)=0.0015 \text{ g/210L}$$

Evaluation of Bias

In this example, bias is evaluated as part of instrument calibration.

The data for the 0.100 g/210 L measurement standard show a difference of the average to the reference value of 0.001 g/210 L. This value is less than the combined standard uncertainty and, therefore, insignificant. No additional component will be added to the measurement uncertainty evaluation.

Step 6—Expand the combined standard uncertainty by coverage factor (k)

The breath alcohol program has 51 measurements of the measurement standard and assumes a lower bound on the effective degrees of freedom (n-1) for the combined standard uncertainty of 50.

The data from the measurement process is assumed to follow a normal distribution; therefore, refer to the Student's t-distribution table to determine the k factor.

For this example, the coverage factor k = 2.05 (n=50) will be used to expand the uncertainty to a 95.45% coverage probability.

$$k=2.05$$

$$U=2.05\times0.0015=0.00308 \text{ g/210L}$$

Step 7—Evaluate the expanded uncertainty

The breath alcohol program determined that the evaluation of uncertainty is fit for purpose.

The breath alcohol program identified that the current method allows for a variance of 0.005 g/ 210L or 5 %, whichever is greater, from a measurement standard known reference value. However, this is greater than the expanded uncertainty at 95.45 %. Left unchanged, a calibration with a significant bias could be reported. Therefore, the breath alcohol program revised the method so that the variability allowed in any calibration must be equal to or less than 0.003 g/ 210L or 3 %, whichever is greater.

Step 8—Report the uncertainty

The breath alcohol program has established a procedure for rounding the expanded uncertainty. Following that procedure, the expanded uncertainty is rounded to the third decimal place to equal the number of decimal places reported in the breath alcohol instrument display. The expanded uncertainty will be 0.003 g/210 L.

The certificate of calibration will contain the following:

— 0.003 g/210 L, the combined expanded uncertainty, rounded to the third decimal place.

— *k* = 2.05, the coverage factor based on the Student's *t*-distribution.

— 95.45 %, the coverage probability

For reporting calibration results, use the rounded expanded uncertainty at the same significance level.

(0.040 g/210 L to 0.300 g/210 L) *± 0.003 g/210 L at a coverage probability of 95.45 % (k=2.05)."*

Uncertainty Budget Form					
Method:	Calibration of breath alcohol measuring instrumentation using long-term calibration data from a single instrument				
Prepared By:	J. Smith		**Date:**	15-Jun-2023	
Sources of Uncertainty	**Type A or B?**	**Std Dev or Outside Limits**	**Distribution Model**	**Divisor**	**Std Uncertainty (1σ)**
Measurement Process Reproducibility (s_r)	A	0.0012	Normal	1	0.0012
Measurement Standards: Uncertainty in Reference Value (u_{CRM})	B	0.0018	Normal	2	0.0009
Combined Uncertainty ($u_c(y)$):	0.0015				
Confidence Level (k):	95.45 % (k = 2.05)				
Expanded Uncertainty (U):	0.00308 **(0.003)**				

Figure C.1: Uncertainty Budget Form-Calibration of breath alcohol measuring instrumentation using long-term calibration data from a single instrument [i]

Annex D
(informative)

Calibration of Breath Alcohol Measuring Instruments Using Control Data from the Calibration Method[n]

Calibration Method Information

A population of breath alcohol measuring instruments is calibrated using the same calibration method with a concentration range of 0.040 g/210 L to 0.300 g/210 L. The calibration method includes multiple measurement standards of varying concentrations and a control. The calibration data obtained is from a population of 100 breath alcohol measuring instruments that have all demonstrated consistent variance across the measurement standard concentration levels. Three measurements of the 0.100 g of ethanol/210 L of breath (g/210 L) control are made during each instrument calibration. Current and historical control data for the population of instruments over time was used in the calculation.

Step 1—Specify the measurement process

Calibration of breath alcohol measuring instruments using control data from the calibration method

Step 2—Identify uncertainty components

The following list of possible contributors to uncertainty in the calibration method was identified:

Personnel

— Inter-personnel variation in performing calibration

— Training

— Experience

Breath Alcohol Measuring Instrument Being Calibrated

— Population of 100 breath alcohol measuring instruments

— Variability of instrument over time

Measurement Standards

— Dry Gas Certified Reference Materials - uncertainty in the stated reference value

[n] An evaluation of measurement uncertainty is specific to the metrological traceability established for the measurement, the measurement assurance processes in place, the breath alcohol program calibration method, the laboratory facility, etc. Therefore, the following is only an example for evaluation and revision by a breath alcohol program after considering the elements specific to that program.

Calibration Method Control

— Dry Gas Certified Reference Material from a different manufacturer than that of the Measurement Standards - uncertainty in the stated reference value

Environmental Conditions

— Barometric pressure

— Humidity

— Temperature

Varying Facilities/Location Change

— Instrument transport

— Power fluctuations

Data Processing

— Processing algorithms

Step 3—Quantify uncertainty components

The breath alcohol program has existing data from the calibration method. Each instrument is evaluated in triplicate using a 0.100 g/210 L dry gas cylinder with metrological traceability as a calibration control. The calibration method requires the control to be within 3 % or 0.003 g/210 L (whichever is greater) of the certified reference value. Furthermore, there shall be no greater than 0.003 g/210 L difference in all three calibration control values.

Control data is collected on an on-going basis with all personnel contributing to the control data for the population of instruments.

Table D.1 shows the individual uncertainty components and how they will be evaluated.

Table D.1—Method of Evaluation of Uncertainty Components

Uncertainty Component	Method of Evaluation
Personnel	
Inter-personnel variation	Adequately represented by Type A Evaluation of process reproducibility data – control
Training	Adequately represented by Type A Evaluation of process reproducibility data – control
Experience	Adequately represented by Type A Evaluation of process reproducibility data – control
Breath Alcohol Measuring Instrument Being Calibrated	
Population of 100 breath alcohol measuring instruments	Adequately represented by Type A Evaluation of process reproducibility data – control
Variability of instrument over time	Adequately represented by Type A Evaluation of process reproducibility data – control
Measurement Standards	
CRM –uncertainty in the stated reference value	Type B Evaluation
Calibration Method Control	
CRM –uncertainty in the stated reference value	Type B Evaluation
Environmental Conditions	
Barometric pressure	Adequately represented by Type A Evaluation of process reproducibility data – control
Humidity	Adequately represented by Type A Evaluation of process reproducibility data – control
Temperature	Adequately represented by Type A Evaluation of process reproducibility data – control
Varying Facilities/Locations	
Instrument transport	Not Applicable
Power fluctuations	Adequately represented by Type A Evaluation of process reproducibility data – control.
Data Processing	
Processing algorithms	Adequately represented by Type A Evaluation of process reproducibility data – control

Type A Evaluation of uncertainty components

Calibration Control Reproducibility – 0.100 g/210 L Calibration Control

The number of measurements of the control in this example is greater than 300.

The statistic that will be calculated is the standard deviation.

To begin, the measurement data's mean (average) and standard deviation will be calculated.[o]

Mean

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

$$\bar{x} = \frac{(x_1 + x_2 + x_3 + \ldots x_n)}{n}$$

The mean of the reproducibility data in this example = 0.0996 g/210 L

Standard Deviation

$$s = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{n-1}}$$

The standard deviation of the reproducibility data in this example = 0.0012 g/210 L

Type B Evaluation of uncertainty components

Certified Reference Materials

The certificates of analysis from all dry gas cylinders were reviewed. The greatest uncertainty is 0.0018 g/210 L for the 0.100 g/210 L CRM.

Step 4—Convert quantities to standard uncertainties

The measurement unit: g of ethanol/210 L of breath (g/210 L)

Type A Evaluation of uncertainty components

Calibration Control Reproducibility – 0.100 g/210 L Calibration Control

The standard deviation of the reproducibility data in this example is 0.0012 g/210 L.

— No additional conversion is necessary to reach a standard uncertainty.

[o] For the readability of the example, the display of digits used in all calculations was abbreviated. The best practice is to include and carry all digits through all calculations and only round the reported value and its uncertainty to the proper number of significant figures.

Type B Evaluation of uncertainty components

Certified Reference Materials

The certificates of analysis state that the expanded uncertainty assumes a normal distribution, a coverage factor of k = 2, and a coverage probability of approximately 95 %.

— The greatest uncertainty is 0.0018 g/210 L.

— The uncertainty on the calibration certificate will be divided by the coverage factor, 2, to arrive at a standard uncertainty.

— 0.0018 g/210 L /2 = 0.0009 g/210 L for the standard uncertainty.

Step 5—Calculate combined standard uncertainty

The evaluation will assume that the uncertainty components are independent or uncorrelated and that the measurement result is the sum of a series of components. The combined standard uncertainty was calculated.

$$u_c(y)=\sqrt{s^2_{\text{reproducibility}}+u^2_{\text{CRMunc}}}$$

$$u_c(y)=\sqrt{0.0012^2_{\text{reproducibility}}+0.0009^2_{\text{CRMunc}}}$$

$$u_c(y)=\sqrt{0.0012^2_{\text{reproducibility}}+0.0009^2_{\text{CRMunc}}}$$

$$u_c(y)=\sqrt{2.25\text{x}10^{-6}}$$

$$u_c(y)=0.0015 \text{ g/210L}$$

Evaluation of Bias

In this example, bias is evaluated as part of the instrument calibration. The calibration method requires the control to be within 3 % or 0.003 g/210 L (whichever is greater) of the certified reference value. Furthermore, there shall be no greater than 0.003 g/210 L difference in all three calibration control values.

The 0.100 g/210 L calibration control data shows a difference between the average and the reference value of 0.001 g/210 L. This value is less than the combined standard uncertainty and, therefore, is insignificant. Although the bias is insignificant, the breath alcohol program chooses to include an additional component in the uncertainty evaluation. An uncertainty contributor equal to the uncertainty of the reference value of the calibration control used for the bias evaluation was added to the evaluation of measurement uncertainty.

Step 3—Quantify uncertainty components – bias component

The breath alcohol program noted that the difference in the average data for the 0.100 g/210 L calibration was 0.001 g/210 L.

Step 4—Convert quantities to standard uncertainties – bias component

The standard uncertainty for the bias was 0.001 g/210 L.

Step 5—Calculate combined standard uncertainty – including bias component

The updated RSS calculation:

$$u_c(y)=\sqrt{s^2_{\text{reproducibility}}+u^2_{\text{CRMunc}}+u^2_{\text{bias}}}$$

$$u_c(y)=\sqrt{0.0012^2_{\text{reproducibility}}+0.0009^2_{\text{CRMunc}}+0.001^2_{\text{bias}}}$$

$$u_c(y)=\sqrt{0.0012^2_{\text{reproducibility}}+0.0009^2_{\text{CRMunc}}+0.001^2_{\text{bias}}}$$

$$u_c(y)=0.0018\ \text{g/210L}$$

Step 6—Expand the combined standard uncertainty by coverage factor (k)

The data from the measurement process is assumed to follow a normal distribution.

The breath alcohol program has 300 calibration control measurements. To determine the k factor, refer to the student's t-distribution table.

For this example, the coverage factor k = 2.0 will expand the uncertainty to a 95.45 % coverage probability.

$$k=2.0$$

$$U=2.0\times0.0018=0.0036\ \text{g/210L}$$

Step 7—Evaluate the expanded uncertainty

The breath alcohol program determined that the evaluation of uncertainty is fit for purpose.

Step 8—Report the uncertainty

The breath alcohol program has established a procedure for rounding the expanded uncertainty. Following that procedure, the expanded uncertainty is rounded to the third decimal place, which will be 0.004 g/210 L.

The certificate of calibration will contain the following:

— 0.004 g/210L, the combined expanded uncertainty, rounded to the third decimal place.

— k = 2.0, the coverage factor based on the Student's t-distribution.

— 95.45 %, the coverage probability

For reporting calibration results, use the rounded expanded uncertainty to the same level of significance

"(0.040 g/210 L to 0.300 g/210 L) *± 0.004 g/210 L at a coverage probability of 95.45 % (k=2.0).*"

Uncertainty Budget Form					
Method:	Calibration of breath alcohol measuring instruments using control data from the calibration method				
Prepared By:	J. Smith		**Date:**	15-Jun-2023	
Sources of Uncertainty	**Type A or B?**	**Std Dev or Outside Limits**	**Distribution Model**	**Divisor**	**Std Uncertainty (1σ)**
Measurement Process Reproducibility (s_r)	A	0.0012	Normal	1	0.0012
Measurement Standards: Uncertainty in Reference Value (u_{CRM})	B	0.0018	Normal	2	0.0009
Bias Component (u_q)	B	0.001	Normal	1	0.001
Combined Uncertainty ($u_c(y)$):	0.0018				
Confidence Level (k):	95.45 % (k = 2.0)				
Expanded Uncertainty (U):	0.0036 **(0.004)**				

Figure D.1: Uncertainty Budget Form-Calibration of breath alcohol measuring instruments using control data from the calibration method [j]

Annex E
(informative)

Bibliography

The following bibliography is not intended to be an all-inclusive list, review, or endorsement of literature on this topic.

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