

2021-S-0004

Standard Practices for

Evaluating Measurement

Uncertainty of Quantitative

Measurements in Forensic

Toxicology

Forensic Toxicology Subcommittee
Toxicology Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

Draft OSAC Proposed Standard

2021-S-0004 Standard Practices for Evaluating Measurement Uncertainty of Quantitative Measurements in Forensic Toxicology

Prepared by
Forensic Toxicology Subcommittee
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**Standard Practices for Evaluating Measurement Uncertainty of Quantitative
Measurements in Forensic Toxicology**

DRAFT

1 **Foreward**

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This document was developed to provide the minimum requirements for evaluating measurement uncertainty for quantitative measurements in forensic toxicology laboratories. 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 measurement traceability. This standard was developed by the Toxicology Subcommittee of the Organizational Scientific Area Committee.

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59 **1 Scope**

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61 This document provides minimum requirements for evaluating measurement uncertainty
62 for quantitative results in forensic toxicology. The document is for testing activities and
63 calibration of breath alcohol measuring instruments and provides direction on evaluation
64 of components, bias, calculations, and reporting. It does not address evaluating
65 measurement uncertainty for breath alcohol testing, this topic will be covered in a different
66 document.

67
68 **2 Normative References**

69
70 National Institute of Standards and Technology, SOP 29-Standard Operating Procedure for
71 the Assignment of Uncertainty (February 2018). Available for download at
72 [https://www.nist.gov/pml/weights-and-measures/laboratory-metrology/standard-](https://www.nist.gov/pml/weights-and-measures/laboratory-metrology/standard-operating-procedures)
73 [operating-procedures](https://www.nist.gov/pml/weights-and-measures/laboratory-metrology/standard-operating-procedures)

74
75 Joint Committee for Guides in Metrology (JCGM) Evaluation of Measurement Data-Guide to
76 the Expression of Uncertainty in Measurement (GUM) (GUM 1995 with minor corrections)
77 (Sevres, France: International Bureau of Weights and Measures [BIPM]-JCGM 100],
78 September, 2008. Available at <https://www.bipm.org/en/publications/guides/gum.html>

79
80 SLR Ellison and A Williams (Eds). Eurachem/CITAC Guide: Quantifying Uncertainty in
81 Analytical Measurement, Third edition, (QUAM: 2012 P1) Available for download at
82 <http://www.eurachem.org/index.php/publications/guides>

83
84 Joint Committee for Guides in Metrology (JCGM), *International vocabulary of metrology –*
85 *Basic and general concepts and associated terms (VIM)*, 3rd ed. (Sèvres, France:
86 International Bureau of Weights and Measures [BIPM]-JCGM 200, 2012) (2008 with minor
87 corrections). Available for download at
88 <http://www.bipm.org/en/publications/guides/vim.html>

89
90 ANSI/ASB Standard 017, Standard Practices for Measurement Traceability in Forensic
91 Toxicology, First Edition, 2018. Available for download at [https://asb.aafs.org/wp-](https://asb.aafs.org/wp-content/uploads/2018/06/017_Std_e1.pdf)
92 [content/uploads/2018/06/017_Std_e1.pdf](https://asb.aafs.org/wp-content/uploads/2018/06/017_Std_e1.pdf)

93
94

95 **3 Terms and Definitions**

96

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

98

99 **3.1**

100 **accuracy**

101 Closeness of agreement between a measured quantity value and a true quantity value of a
102 measurement.

103

104 **3.2**

105 **analytical run (batch)**

106 A set of standards, controls, and/or case samples that are contemporaneously prepared
107 and/or
108 analyzed in a particular sequence.

109

110 **3.3**

111 **bias, statistical**

112 A systematic tendency for estimates or measurements to be above or below their true
113 values.

114

115 Note 1: Statistical bias arises from systematic as opposed to random error.

116

117 Note 2: Statistical bias can occur in the absence of prejudice, partiality, or discriminatory
118 intent.

119

120 **3.4**

121 **calibration**

122 Operation that, under specified conditions, establishes a relationship between the quantity
123 value and corresponding indications.

124

125 **3.5**

126 **calibrator**

127 Measurement standard used in calibration.

128

129 **3.6**

130 **certified reference material**

131 **CRM**

132 Reference material (RM) characterized by a metrologically valid procedure for one or more
133 specified properties, accompanied by a certificate that provides the value of the specified
134 property, its associated uncertainty, and a statement of metrological traceability.

135

136 **3.7**

137 **control**

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

140
141 **3.8**
142 **limit of detection**
143 **LOD**
144 An estimate of the lowest concentration of an analyte in a sample that can be reliably
145 differentiated from blank matrix and identified by the analytical method.
146
147 **3.9**
148 **lower limit of quantitation**
149 **LLOQ**
150 An estimate of the lowest concentration of an analyte in a sample that can be reliably
151 measured with acceptable bias and precision.
152
153 **3.10**
154 **measurand**
155 The quantity intended to be measured.
156
157 **3.11**
158 **measurement traceability**
159 **(metrological traceability)**
160 Property of a measurement result whereby the result can be related to a reference through
161 a documented unbroken chain of calibrations, each contributing to the measurement
162 uncertainty.
163
164 **3.12**
165 **precision**
166 The measure of the closeness of agreement between a series of measurements obtained by
167 replicate measurements on the same or similar samples.
168
169 **3.13**
170 **repeatability**
171 Measurement precision under a set of conditions that includes the same measurement
172 procedure, same operators, same measuring system, same operating conditions, same
173 conditions and same location, and replicate measurements on the same or similar objects
174 over a short period of time.
175
176 **3.14**
177 **reproducibility**
178 Measurement precision under a set of conditions that includes different locations,
179 operators, measuring system, and replicate measurements on the same or similar objects.
180
181
182
183

184 **4 Measurement Uncertainty**

185

186 **4.1 Background**

187

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

194

195 Measurement Uncertainty (MU) is an estimate of the potential variability of a measurement
196 based on the information known about the measurand and the measurement method. The
197 measurement may be part of the test, a calibration method, or the final reported test or
198 calibration result. “Measurement uncertainty does not imply doubt about the validity of a
199 measurement; on the contrary, knowledge of the uncertainty implies increased confidence
200 in the validity of the measurement result¹”

201

202 Laboratory stakeholders require tests and calibrations performed to be reliable, accurate,
203 and comparable. MU is an important parameter describing the confidence, as well as
204 limitations, of measurement results. Comparison of quantitative test or calibration results
205 between laboratories or evaluation of quantitative results in relation to a legal specification
206 or requirement necessitates knowledge of the MU.

207

208 The National Institute for Standards and Technology (NIST) has developed an 8-step
209 process for evaluating and reporting MU (Figure 1).² This framework established by NIST
210 conforms to the principles set forth in the Joint Committee for Guides in Metrology (JCGM)
211 Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement
212 (GUM³) and is a helpful reference.

213

214

215

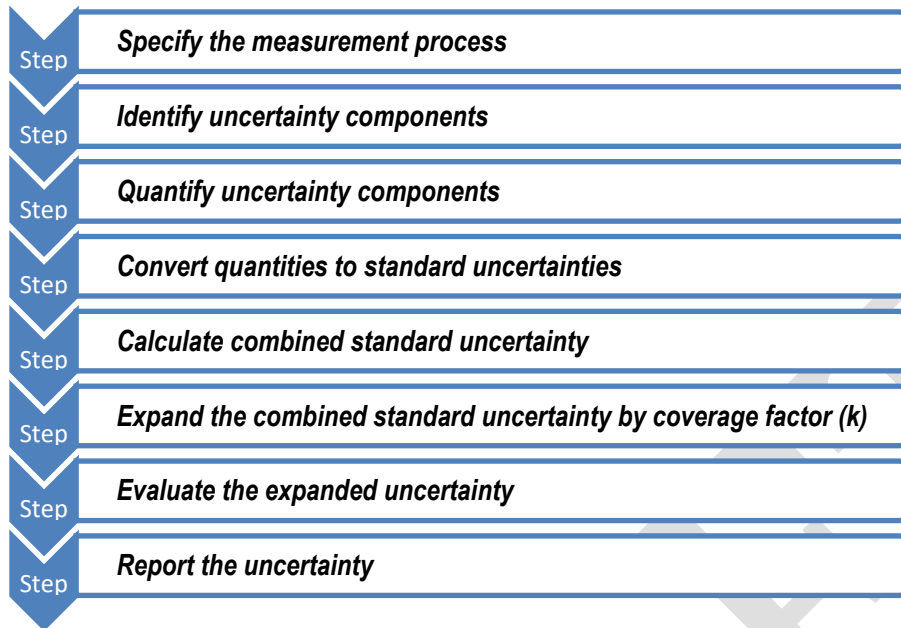
216

¹ SLR Ellison and A Williams (Eds). Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (QUAM: 2012 P1) Available for download at <http://www.eurachem.org/index.php/publications/guides>

² National Institute of Standards and Technology, SOP 29-Standard Operating Procedure for the Assignment of Uncertainty (April 2021). Available for download at <https://www.nist.gov/system/files/documents/2019/05/13/sop-29-assignment-of-uncertainty-20190506.pdf>

³ 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 at <http://bipm.org/en/publications/guides/gum.html>

217 Figure 1: The NIST 8-Step Process for Evaluating and Reporting Measurement Uncertainty⁴
218



219
220

221 **4.2 Requirements for Measurement Uncertainty for Quantitative Determinations**

222

223 **4.2.1 General Requirements**

224

225 4.2.1.1 Laboratories shall have and apply procedures for evaluating MU for methods used
226 to calibrate breath alcohol instruments and for test methods that produce a quantitative
227 test result.

228

229 4.2.1.2 MU is specific to each measurement process and shall be evaluated separately for
230 each analyte in each testing or calibration method.

231

232 In testing, this requires that each combination of analyte, extraction and analytical
233 technique be evaluated separately. Multiple matrices may have to be evaluated separately
234 based on results of method validation.

235

236 4.2.1.3 Using the largest evaluated MU for more than one analyte within a method or one
237 analyte across methods is not acceptable.

238

239 4.2.1.4 Test and Calibration Methods for which the MU is evaluated shall meet the
240 minimum requirements set forth in:

241

⁴ Adapted from ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty-Annex D
Note: Document can be obtained from anab@anab.org

- 242 a. *ANSI/ASB Standard 017*, Standard Practices for Measurement Traceability in Forensic
243 Toxicology.
244 b. *ANSI/ASB Standard 036*, Standard Practices for Method Validation in Forensic
245 Toxicology
246

247 **4.2.2 Step 1: Specify the Measurement Process**

248

249 The measurand shall be defined and documented. This can be in the form of a written
250 statement, a visual diagram, and/or a mathematical expression.
251

252 NOTE: To be clear about the measurement process for which the MU evaluation is for, it is
253 important to be as specific as possible when defining the measurand. To distinguish one
254 measurement process from another within a laboratory, it may be necessary to include a
255 reference to a specific type of equipment used or a specific procedure in the statement
256 defining the measurand.
257

258 EXAMPLES:

259 *Testing of biological samples*

- 261 Concentration of ethanol (g/100mL) in ante-mortem whole blood
262 Concentration of oxycodone (mg/kg) in a sample of liver homogenate
263

264 *Calibration of breath alcohol measuring instruments*

- 265 Calibration of XYZ model breath alcohol measuring instrument using dry gas certified
266 reference material
267

268 **4.2.3 Step 2: Identify Uncertainty Components**

269

270 Minimum method components that shall be considered, as applicable, in an evaluation
271 of MU include:
272

- 273 a. Certified reference material(s) and calibrations of equipment used to establish
274 measurement traceability;
275
276 b. Data from the measurement process (i.e. repeatability, reproducibility or from
277 intermediate measurement conditions)
278
279 c. Human factors (e.g., multiple analysts performing the same measurement method,
280 experience, training, etc.);
281
282 d. Sampling conducted during the measurement method;
283
284 e. Sample preparation; and
285
286 f. Environmental conditions during the measurement process.

287
288

289 **4.2.4 Step 3: Quantify Uncertainty Components**

290

291 Uncertainty components shall be quantified. All digits shall be carried through calculations
292 until final expanded measurement uncertainty is determined. Only then should rounding
293 and significant figure rules be applied.

294

295 The GUM (2.3.2) refers to the method of evaluation of one or more uncertainty components
296 as:

297

298

299

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 quality control results)

300

301

302

303

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)

304

305

306

307

308

The method of evaluation, Type A or Type B, will be determined for each component
identified. It is most common to have a mixture of the two methods where some identified
uncertainty components are quantified using a Type A method of evaluation and some
identified uncertainty components are quantified using a Type B method of evaluation.

309

310

311

312

Any double-counting of a component will result in an overestimation of the measurement
uncertainty and should be avoided, when possible. However, overestimation is generally
more desirable than underestimation.

313

314

A record shall be maintained for *Type A* and *Type B* evaluations.

315

316

4.2.4.1 Minimum requirement(s) for data used in *Type A* evaluations:

317

318

319

4.2.4.1.1 Shall come from method validation and/or ongoing quality control
(measurement assurance program) for the measurement method.

320

321

322

a. Method validation may include the evaluation of one or more specific uncertainty
components.

323

324

325

326

b. Data from proficiency tests may only be used if the proficiency test has established
metrological traceability for the quantitative value of the proficiency test. A consensus
value does not establish metrological traceability.

327

328

329

4.2.4.1.2 Shall be representative of the measurand that will be tested or calibrated.

330

331

4.2.4.1.3 Shall be representative of the range (e.g., matrix, or detector response over the
expected concentration range, etc.) of the measurements made.

332
333 **4.2.4.1.4** Shall be evaluated according to the size and distribution of the statistical sample.
334

335 **4.2.4.2 Establishing a quantity value for *Type A* evaluations**
336

337 To appropriately evaluate the magnitude of uncertainty for the measurement process using
338 *Type A* evaluation, calculate a standard deviation or a relative standard deviation using
339 historical data for each identified *Type A* uncertainty component. Typically, method
340 performance is best represented by measurements of quality control (QC) samples taken
341 over multiple instrumental batches, each with different instrument calibrations (cf.
342 ANSI/ASB Standard 036, 2019). A graphical representation of all QC measurements used
343 for the *Type A* uncertainty component that demonstrates statistical control of the
344 measurements used shall be maintained. Additional methods may also be used to ensure
345 statistical control.
346

347 If multiple QC measurements are available in each instrumental batch, all QC
348 measurements can be included when computing the standard deviation or relative
349 standard deviation. Inclusion of multiple QC measurements in the computation of the
350 standard deviation will bias the standard uncertainty estimate slightly if the QC data
351 exhibits any batch-to-batch variation but mitigates the need for more complex standard
352 deviation computations. If needed, other statistical methods, such as the ANOVA method
353 outlined in section 8.2.2.3.4 of ANSI/ASB Standard 036 or random subsampling of the QC
354 data to select a single representative QC measurement from each batch, can be used to
355 correct for this bias.
356

357 If the result to be reported for a specimen will be either an individual measured value or
358 the average of multiple measured values from a single instrumental batch, the standard
359 deviation or the relative standard deviation shall be used as the *Type A* standard
360 uncertainty for the reported specimen value. Setting aside the slight bias produced if the
361 standard deviation is computed from data containing multiple QC measurements in each
362 batch, this standard uncertainty should provide an assessment of the *Type A* uncertainty
363 that is either on target or conservative (i.e., larger than necessary) for the reported
364 specimen value.
365

366 If the the result to be reported for a specimen will be the average of measured values from
367 multiple instrumental batches, the standard deviation or the relative standard deviation
368 divided by the square root of the number of instrumental batches used when averaging the
369 specimen data shall be used as the *Type A* standard uncertainty for the reported specimen
370 values. Division by the square root of the number of batches converts the standard
371 deviation for single-batch results into the standard deviation of the mean of multiple-batch
372 results. As above, setting aside the slight bias produced if the standard deviation is
373 computed from data containing multiple QC measurements in each batch, this standard
374 uncertainty should provide an assessment of the *Type A* uncertainty that is either on target
375 or conservative for the reported specimen value.
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4.2.4.2.1 Testing Laboratories

4.2.4.2.1.1 Use of Validation Data

Validation data may initially be used for the Type A uncertainty component. Continued use of validation data for this uncertainty component requires that laboratories demonstrate the data is representative of the data generated during day-to-day analysis by analysts who have demonstrated competence.

4.2.4.2.1.2 Multiple controls within the same method

For methods where validation has demonstrated constant variance across the entire calibration range (homoscedasticity) as shown through the use of residual plots for the calibration curve or other statistical means, laboratories shall use either:

- a. Combined data from all controls analyzed; or
- b. Select data from one specified control (e.g., a control at or near a legal specification).

For methods where validation has demonstrated that variance is not constant across the entire calibration range (heteroscedasticity), laboratories shall establish a procedure for how MU will be calculated. Procedures may include:

- 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 changes occur across the calibration range and establish an appropriate uncertainty to report based on where these variation changes occur; or
- c. Utilize the Type A data from the control at the concentration closest to the sample concentration. This is acceptable only when an evaluation of the difference in the standard deviation between the two applicable control levels does not impact the evaluation of conformance with a legal specification.

4.2.4.2.1.3 Multiple Analysts/Instruments/Laboratories

For a method that has been validated on multiple instruments or in multiple laboratories by analysts who have demonstrated competence, provided that quality control criteria for acceptance and reporting criteria are the same across all instruments and laboratories, calculate MU using control data in accordance with Section 4.2.4.2.1.4..

422 **4.2.4.2.1.4 Quality Control Data**

423

424 Appropriate methods for calculating MU using quality control data include, but are not
425 limited to:

426

427 a. Calculation of MU using control data generated since validation or first day-to-day use
428 of the method;

429

430 b. Calculation of a rolling MU where the laboratory chooses to include a set number of
431 data points from the most recent analyses. The data shall be representative of the
432 performance of the method; or

433

434 c. Calculation of a batch-specific MU based on use of data from only the current analytical
435 batch. This method is more commonly used for non-routine analysis where limited data
436 points are available.

437

438 **4.2.4.2.2 Calibration of Breath Alcohol Measuring Instruments**

439

440 **4.2.4.2.2.1 Use of Validation Data**

441

442 Validation data may initially be used for the Type A uncertainty component. Continued use
443 of validation data for this uncertainty component requires that laboratories demonstrate
444 that the data is representative of the data generated during day-to-day calibration of breath
445 alcohol measuring instruments by personnel who have demonstrated competence.

446

447 **4.2.4.2.2.2 Multiple measurement standards within the same method**

448

449 For methods that have demonstrated constant variance across the entire calibration range
450 as shown through the use of residual plots for the calibration curve or other statistical
451 means, laboratories may either:

452

453 a. Combine data from all measurement standards analyzed to estimate a single MU; or

454

455 b. Calculate the measurement uncertainty at each measurement standard concentration.

456

457 For methods where validation has demonstrated that variance is not constant across the
458 entire calibration range, laboratories may either:

459

460 a. Perform an in-depth evaluation to determine where the variance changes occur across
461 the calibration range and establish an appropriate uncertainty to report based on
462 where these variance changes occur; or

463

464 b. Calculate the MU at each measurement standard concentration across a population of
465 instruments or for an individual breath alcohol measuring instrument.

466

467 **4.2.4.2.2.3 Use of Measurement Standard Data or Quality Control Data**

468
469 Appropriate methods of selecting measurement standard data or quality control data
470 include, but are not limited to, the following across a population of instruments or for an
471 individual instrument:

- 472
473 a. Calculation of MU using measurement standard data generated since validation or first
474 day-to-day use of the instrument; or
475
476 b. Calculation of a rolling MU where the laboratory chooses to include a set number of
477 data points. The data shall be representative of the performance of the instrument.
478

479 **4.2.4.3 Minimum requirements for *Type B* evaluations:**

480
481 Components requiring a *Type B* evaluation may include: uncertainty associated with a
482 certified reference material, uncertainty of a reference material, and/or uncertainty from
483 equipment calibration (e.g., balance, volumetric flask, pipette, barometer, or thermometer).
484

485 **4.2.4.3.1** Shall consider all components that are not accounted for in a *Type A* evaluation.
486

487 **4.2.4.3.2** Shall account for all identified and significant systematic bias (see 4.2.6.1).
488

489 **4.2.4.3.3** Shall be handled according to the assumed distribution of the quantity value.
490

491 **4.2.4.4 Establishing a quantity value for *Type B* evaluations**

492
493 **4.2.4.4.1** For component(s) used in the preparation of a calibrator, the components can be
494 quantified individually or as a group for the calibrator.

- 495
496 a. If estimating uncertainty over the full calibration range, use the largest standard
497 deviation calculated above;
498
499 b. If estimating the uncertainty for multiple concentration ranges, use the largest standard
500 deviation calculated above for each concentration range, respectively;
501
502 c. If estimating the uncertainty at each calibrator or measurement standard concentration
503 separately, use the value for the applicable calibrator.
504

505 If the test or calibration method includes the preparation of multiple calibrators or
506 measurement standards, the individual components can be quantified individually across
507 all calibrator concentrations (e.g. a single component quantity value can be used for the
508 pipette uncertainty that adequately covers the pipettes used to prepare all calibrator
509 concentrations) and then a or b below can be applied. Alternatively, the components can be
510 quantified as a group for each calibrator concentration and then a - c applied.
511

512 Depending on the measurement process, these components related to calibrator
513 preparation, typically requiring a *Type B* evaluation, may be accounted for by on-going
514 quality control data (*Type A*).

515
516 **4.2.5 Step 4: Convert Quantities to Standard Uncertainties**

517 Quantify all uncertainty components as a standard uncertainty of the quantity values and in
518 the same measurement unit or in a measurement unit relative to the quantity values.

519
520 **4.2.5.1 Type A evaluations**

521
522 Typically, an assessment of Type A uncertainty is calculated to be a standard uncertainty. If
523 not already presented as a standard uncertainty, divide by the appropriate factor (*e.g.*, 2 or
524 3) to convert to a standard uncertainty.

525
526 **4.2.5.2 Type B evaluations**

527
528 If not reported by the manufacturer as a standard uncertainty, the appropriate probability
529 density function for the component needs to be used to compute one standard deviation or
530 relative standard deviation associated with the specified distribution.

531
532 If reported by the manufacturer as an expanded uncertainty, divide by the appropriate
533 coverage factor (*e.g.*, 2 or 3), to arrive at a standard uncertainty.

534
535 **4.2.6 Step 5: Calculate the combined standard uncertainty**

536
537 Calculate the combined standard uncertainty using each uncertainty contributor quantity
538 value. Acceptable methods to do so include the root sum of the squares formula and the
539 Monte Carlo⁵ method.

540
541 After the combined standard uncertainty is calculated, components may be individually
542 evaluated for significance. A component is deemed significant if it impacts the least
543 significant digit in the reported value for MU.⁶ Components determined to be insignificant
544 may be removed from the uncertainty calculations.

545
546 Note: If multiple individual components are removed from the uncertainty combination,
547 then the aggregate impact of the removed components should be evaluated.

⁵ 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 at https://www.bipm.org/utis/common/documents/jcgm/JCGM_101_2008_E.pdf

⁶ National Institute of Standards and Technology, SOP 29-Standard Operating Procedure for the Assignment of Uncertainty (February 2018). Available for download at <https://www.nist.gov/pml/weights-and-measures/laboratory-metrology/standard-operating-procedures>

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4.2.6.1 Evaluation of bias⁷

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. An evaluation of bias requires one or more controls prepared with metrological traceability, having a known reference value and uncertainty.

4.2.6.1.1 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. Estimate the combined uncertainty without considering the relevant bias.
- c. Compare the bias with the combined standard uncertainty.
 - i. Where the bias is less than the combined standard uncertainty, $bias < u_c$, the bias is viewed as not significant and may be neglected or included as a component in the estimation of uncertainty.
 - ii. Where the bias is greater than the combined standard uncertainty, $bias > u_c$, it is viewed as significant and additional action is required, see 4.2.6.1.1.1 and 4.2.6.1.1.2.

4.2.6.1.1.1 Testing

- a. Eliminate or reduce the bias until it is not significant; or
- 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 estimation of MU shall be reported; or
- c. Report the measurement result and the expanded MU with bias included. The method used to include the bias in the expanded uncertainty shall be a statistically valid method⁷ and in compliance with the GUM; or
- d. Report the observed measurement result, the MU, and the bias.

⁷ Section 3.2.5 of NIST SOP 29 (2019)

591 **4.2.6.1.1.2 Calibration of Breath Alcohol Instruments**

592
593 Eliminate or reduce bias until it is not significant by repeating the adjustment process
594 and/or performing the appropriate repair.

595
596 **4.2.7 Step 6: Calculate the expanded uncertainty**

597
598 **4.2.7.1** A coverage factor (k) shall be determined using a Student's t-distribution⁸ based
599 on the degrees of freedom to provide the desired level of confidence.

600
601 **4.2.7.2** The minimum coverage probability for all quantitative test results and calibration
602 results shall be 95.45 % (often referred to as approximately 95 %).

603
604 **4.2.8 Step 7: Evaluate the expanded uncertainty**

605
606 **4.2.8.1** A determination whether the evaluated measurement uncertainty is acceptable
607 shall be made by the laboratory. The laboratory is responsible for supporting their
608 decision. As applicable, minimum aspects to consider include:

- 609
610 a. Stakeholder interests;
611
612 b. Legal requirements;
613
614 c. The relationship between the reported test or calibration quantitative value and the
615 expanded MU; particular consideration shall be taken around the LLOQ/LOD (e.g., an
616 expanded MU of 0.01 ng/mL for a method with an LLOQ of 0.01 ng/mL should prompt
617 the laboratory to reevaluate the LLOQ.); and
618
619 d. The relationship between the quality control limits for the method and the expanded
620 measurement uncertainty (e.g., ± 20 % quality control limits for a method with
621 expanded MU of 10 %. For any single analytical batch, this QC limit would allow a
622 variation of up to 20% which exceeds the stated expanded MU for the method. This
623 should prompt the laboratory to reevaluate the quality control limits to ensure the MU
624 statement will always be correct).

625
626 **4.2.9 Step 8: Report the expanded uncertainty**

627
628 **4.2.9.1** The estimated MU shall be included in the test/calibration report or an attachment
629 to the report for all quantitative test results in accordance with the *ANSI/ASB Standard 053*
630 *Standard for Reporting in Forensic Toxicology* and for all calibrations.

- 631
632 a. The MU shall be reported as an expanded uncertainty and include the coverage
633 probability for testing laboratories

⁸ Table G2 of the GUM, another reference table, or appropriate statistical software

- 634
635 b. The MU shall be reported as an expanded uncertainty and include the coverage factor,
636 k, and the coverage probability for calibration laboratories.
637
638 c. The measurement result shall include the measured quantity value, y , along with the
639 associated expanded uncertainty, U , and the measurement result should be reported as
640 $y \pm U$ where U is consistent with the units of y . Specific applications may warrant use of
641 a different format than $y \pm U$.
642
643 d. The expanded uncertainty should be reported to at most 2 significant figures unless the
644 laboratory has a documented rationale to report beyond 2 significant figures.
645
646 e. Rules for rounding the expanded uncertainty shall be defined by the laboratory.
647
648 f. The measurement result shall be reported using the same number of decimal places as
649 the rounded expanded uncertainty unless a legal specification specifies how the
650 measurement result is to be reported. Rules for rounding or truncating the
651 measurement result shall be defined by the laboratory.
652
653 g. Laboratories shall not report the single largest measurement uncertainty for a group of
654 analytes within a method or the largest measurement uncertainty for a single analyte
655 across multiple methods.
656
657 h. For testing laboratories, if a significant bias is identified and the action taken is
658 4.2.6.1.1.1 b or c, this shall be clearly communicated.
659

660 **4.3 Periodic evaluation of measurement uncertainty**

661
662 The interval for review and recalculation of a method's MU shall be set by the laboratory.
663 The interval and re-evaluation of measurement uncertainty will depend on, but not be
664 limited to, the following factors:

- 665
666 a. Both Type A and Type B uncertainty components included in the calculation;
667
668 b. The frequency with which one of the components change;
669
670 c. The frequency with which the testing or calibration method is performed;
671
672 d. The magnitude of a change in a component in relationship to the calculated MU;
673
674 e. A change in the measurement process; and
675
676 f. Any laboratory administrative decision such as a set time interval.
677

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

DRAFT

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ANNEX A
(Informative)

Concentration of ethanol in an ante-mortem blood specimen⁹

Test Method Information

Multiple analysts were trained and qualified to use the laboratory’s method to determine the concentration of ethanol in ante-mortem blood specimens. All analysts 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 gas chromatography with a flame ionization detector. Samples are introduced to the gas chromatograph via a headspace autosampler.

Calibrators are used to generate a calibration curve with each analytical batch. The calibrators 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 prior to use (i.e., not diluted). Constant variance (homoscedasticity) was observed across the concentration range. Method validation indicated that the proper calibration model was unweighted linear regression.

Measurement assurance is achieved through the use of Quality Control (QC) samples. These include a quantitative blood matrix control prepared by the laboratory at approximately 0.080 g/dL and unaltered 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 prior to use.

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.

Measurement Traceability

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

⁹ An evaluation of measurement uncertainty is specific to the measurement traceability that has been established for the measurement, the measurement assurance processes that are in place, the laboratory test method, the laboratory facility, etc. Therefore, the example that follows shall be evaluated and revised by each laboratory to take into consideration the elements that are specific to that laboratory.

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

724
725 All external calibrations of measuring equipment are performed by calibration laboratories
726 that meet the *ANSI/ASB Standard 017, Standard Practices for Measurement Traceability in*
727 *Forensic Toxicology.* The pipette diluter has been and is routinely calibrated.

728
729 **Measurement Assurance**

730
731 The quantitative blood matrix control is prepared by the laboratory to a concentration of
732 approximately 0.080 g/dL. It is made in a large batch, packaged, and stored in a manner to
733 provide a long shelf-life for the control. The expected concentration is determined in-house
734 through repeat measurements. Pre-defined criteria for acceptable performance are based
735 on historical data across multiple lots from the last 2 years. To date, the laboratory has
736 greater than 100 measurements made using this control since the method was validated.

737
738 The CRMs used for QC samples at low, medium, and high concentrations were purchased
739 from a different supplier than the CRMs used as calibrators.

740
741 The QC samples are used to ensure validity of the test method across the concentration
742 range. The CRM QC samples are also used to verify the calibration curve and to evaluate the
743 method's bias on an ongoing basis.

744
745 **Step 1 Specify the measurement process**

746
747 As a written statement:

748
749 *"The Concentration of Ethanol in Ante-Mortem Blood using [the validated laboratory*
750 *procedure]"*

751
752 **Step 2 Identify uncertainty components**

753
754 The following list of *possible* contributors to the uncertainty in this method were identified
755 by the laboratory:

756
757 Analyst

- 758 ● Inter-analyst variation in sample preparation and measurements
759 ● Training
760 ● Experience

761
762 Calibrators

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

765

766 Quality Control Samples

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

769

770 Internal Standard Preparation

- 771 ● Components:
772 ● NaCl – reagent grade
773 ● n-propanol – reagent grade
774 ● Concentration – equipment used to prepare (balance, volumetric flask)

775

776 Preparation of aliquots of Calibrators, Quality Control Samples and Measurand

- 777 ● Homogenization
778 ● Test Specimens – mixing
779 ● Matrix control – mixing
780
781 ● Temperature
782 ● All calibrators, quality control samples and the test specimens are brought to room
783 temperature
784 ● Variation in the time allowed to reach room temperature
785 ● Variation in room temperature at different times of year
786
787 ● Pipette diluter
788 ● Volume of sample and volume of internal standard
789 ● Calibration uncertainty or laboratory specification to verify calibration status
790
791 ● Headspace vials
792 ● Crimping action
793 ● Material of vial and stopper
794
795 ● Time between replicate sampling of test specimens

796

797 Analysis

- 798 ● Instrument parameter settings (e.g., oven temperature(s), gas flow, split ratio, aging of
799 chromatographic column, autosampler syringe, autosampler precision, headspace
800 equilibration time, headspace equilibration temperature, etc.)
801 ● Interference from the matrix
802 ● Interference from reagents
803 ● Interference from other compounds
804 ● Stability of sample(s) from preparation through analysis
805 ● Instrument precision
806 ● Systematic instrumental variation within an analytical batch

807

808 Data Processing

- 809 ● Calibration model
810 ● Integration parameters

- 811 • Processing algorithms

812

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

815

816 NOTE: A laboratory may identify different uncertainty components when an evaluation of
817 their specific measurement process is performed.

818

819 **Step 3 Quantify uncertainty components**

820

821 The laboratory has existing data from the measurement process:

822

- 823 • The calibration model was determined during method validation and was shown
824 through the use of residual plots to have constant variance across the linear range.
825 Therefore, the laboratory is going to evaluate a single measurement uncertainty to
826 represent the entire reportable concentration range.

827

- 828 • Each analytical batch does include one or more independently-prepared samples of the
829 blood matrix quality control sample. This blood matrix QC sample is prepared to have
830 an ethanol concentration of approximately 0.080 g/dL. All analysts have made
831 measurements using this blood matrix QC sample (across multiple lots). To date, the
832 laboratory has greater than 100 measurements of the blood matrix QC sample since
833 validation.

834

- 835 • The laboratory also has data from three certified reference materials that were used as
836 quality control samples. The ethanol concentration of the CRM QC samples spans the
837 reportable concentration range. The primary use of the CRM QC samples is to evaluate
838 bias in the measurement method, but these samples also provide additional evaluation
839 of a number of uncertainty components.

840

841 Table 1 shows the individual uncertainty components and how they will be evaluated:

842

843 *Table 1: Method of Evaluation of Uncertainty Components (Example 1)*

Uncertainty Component	Method of Evaluation
Analysts	
Inter-analyst variation	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).
Training	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).
Experience	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).
Calibrators	

CRM – uncertainty in the stated reference value	<i>Type B Evaluation</i>
Matrices of calibrators and test specimens	Initially evaluated during method validation and determined to be insignificant, therefore not included in the uncertainty evaluation.
Quality Control Samples	
CRM – second source; uncertainty in the stated reference value	Primary use is to evaluate bias. The evaluation of bias will be done after the calculation of combined standard uncertainty.
Matrix control - stability	Adequately represented by the <i>Type A Evaluation</i> 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, Quality Control Samples and Test Specimens	
Homogenization – mixing	Initially evaluated during method validation and determined to be significant, therefore controlled through the procedure administrative requirement for agreement of replicates (<i>Type B Evaluation</i>).
Temperature – all calibrators, quality 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 year	Partially quantified in <i>Type A Evaluation</i> of process reproducibility data - blood matrix QC sample and partially through the procedure administrative requirement for agreement of replicates (<i>Type B Evaluation</i>).
Pipette diluter: Volume of sample, volume of internal standard and dilution Calibration uncertainty or laboratory specification to verify calibration status	<i>Type B Evaluation</i>
Pipette diluter: Variation in use by multiple staff	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).
Headspace vials: Crimping Material of stopper	Adequately represented by the <i>Type A Evaluation</i> 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 (<i>Type B Evaluation</i>).
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 <i>Type A Evaluation</i> 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 quality 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 <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample) and through the procedure administrative requirement for agreement of replicates.
Instrument precision	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).
Systematic instrumental variation within an analytical batch	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample) and partially through the procedure administrative requirement for agreement of replicates (<i>Type B Evaluation</i>).
Data Processing	
Calibration model	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample and CRMs used as QC).
Integration parameters	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).
Processing algorithms	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data (Blood Matrix QC Sample).

844
845
846
847
848
849

850 **Type A Evaluation of uncertainty components**

851

852 **Measurement Process Reproducibility - Blood Matrix quality control sample**

853

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

856

857 To begin, the mean (average) and standard deviation of the blood matrix QC sample values
858 will be calculated.¹⁰

859

860 The mean is calculated as:

861

862
$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i = \frac{(x_1 + x_2 + x_3 + \dots + x_n)}{n}$$

863

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

865

866 The standard deviation is calculated as:

867

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

868

869

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

871

872 Relative Standard Deviation (RSD) is calculated as:

873

874
$$RSD = \frac{s}{\bar{x}}$$

875

876
$$\% RSD = RSD \times 100 \%$$

877

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

879

880
$$RSD = \frac{0.0027 \text{ g/dL}}{0.0798 \text{ g/dL}} = 0.0341$$

881

882
$$\% RSD = 0.0341 \times 100 = 3.41 \%$$

883

884 **Type B Evaluation of uncertainty components**

¹⁰ 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.

885

886 **Interference from the matrix**

887

888 The laboratory did evaluate matrix effects during method validation which resulted in the
889 test method incorporating a dilution factor using the pipette diluter. Dilution of the sample,
890 in combination with the procedural requirements to mix the test item minimizes matrix
891 effects. The laboratory does acknowledge that it is impossible to evaluate all variations in
892 test item matrix during method validation; therefore, the test method does include a blood
893 matrix QC sample and a requirement for agreement between replicate samples to quantify
894 the impact of matrix on the measurement.

895

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

903

904 The laboratory procedure requires that two aliquots be taken from the homogenized test
905 item and that the measured ethanol concentrations of the two aliquots must be within ± 5
906 % of the average or the analysis is repeated.

907

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

915

916 **Calibrators: Uncertainty in the reference value**

917

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

920

$$921 \quad \text{Relative uncertainty} = \left(\frac{0.000233 \text{ g/dL}}{0.010 \text{ g/dL}} \right) * 100 = 2.33 \%$$

922

923 **Pipette Diluter**

924

925 The laboratory has set internal criteria (± 3 %) to ensure proper functioning of the pipette
926 diluter. This is greater than the specifications for calibration used by the external
927 calibration laboratory (± 2 %). Additionally, the procedure to ensure proper functioning is
928 performed quarterly compared to the external calibration which is performed annually.

929 Therefore, the laboratory criteria of $\pm 3\%$ will be used to quantify variability for this
930 uncertainty component.

931

932 **Step 4 Convert quantities to standard uncertainties**

933

934 **The measurement unit**

935

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

937

938 **Type A Evaluation of uncertainty components**

939

940 **Measurement Process Reproducibility data**

941

942 Test specimens are sampled in duplicate, analyzed in two separate batches and the
943 laboratory procedure for the reported ethanol concentration is to average the two results.
944 Repeat measurements of the test specimens provide more information and more
945 confidence that the reported result is the best estimate of the true value. The measurement
946 process reproducibility data is based on single measurements of 0.08 g/dL blood matrix QC
947 sample. Therefore, the %RSD of the mean is calculated by taking the %RSD of the
948 measurement process and dividing by the square root of the number of measurements
949 averaged to generate the reported ethanol concentration.

950

951 NOTE: If a single measurement result for the test specimens is selected to be reported (e.g.,
952 the lowest value), then the standard deviation of the mean calculation is not applicable.

953

954 NOTE: If the laboratory makes an equal number of multiple measurements of the quality
955 control sample as it does of the test specimens and averages the results to evaluate the
956 acceptability of the quality control sample, then the standard deviation of the mean
957 calculation is not applicable.

958

959 The %RSD of the reproducibility data in this example is 3.41 %

960

961 The mathematical expression for %RSD of the mean:

962

$$963 \quad \%RSD_{mean} = \frac{\%RSD}{\sqrt{n}}$$

964

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

966

$$967 \quad \%RSD_{mean} = \frac{3.41\%}{\sqrt{2}} = 2.4101\%$$

968

969

970

971

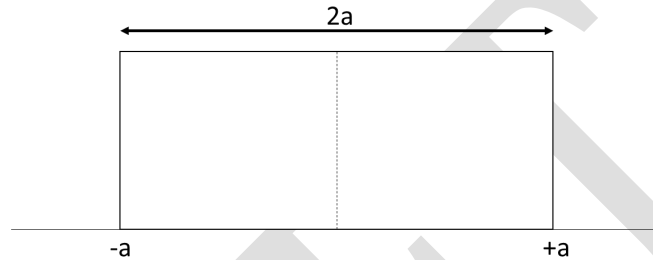
972 **Type B Evaluation of uncertainty components**

973

974 **Interference from the matrix**

975

976 The laboratory procedure requires two samples to be taken from the homogenized test
977 specimens and the ethanol concentration of the two aliquots to be within $\pm 5\%$ of the
978 average, or the analysis is repeated. This component is evaluated as a rectangular
979 distribution:
980



981

982

$$\text{Upper limit} = +a$$

983

$$\text{Lower limit} = -a$$

984

$$\text{Possible range of values} = (+a) - (-a) = 2a$$

985

986

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

988

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

989

990

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

993

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

994

995

996 **Calibrators: Uncertainty in the reference value**

997

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

1000

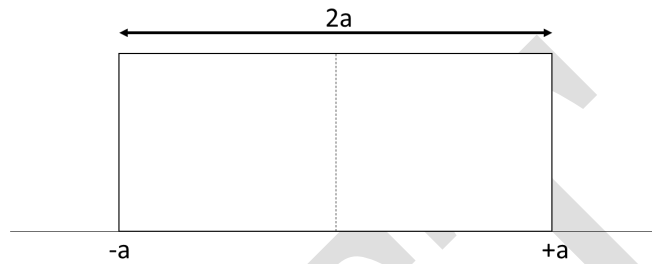
1001 The certificate indicates this expanded uncertainty assumes a normal distribution, a
1002 coverage factor of $k = 2$, and a coverage probability of approximately 95 %. The uncertainty
1003 on the calibration certificate will be divided by the coverage factor to arrive at a relative
1004 standard uncertainty.
1005

1006

$$\text{Relative standard uncertainty} = \left(\frac{2.33\%}{2} \right) = 1.165\%$$

1007
1008 **Pipette Diluter**

1009
1010 In Step 3, the laboratory determined that its in-house criteria of $\pm 3\%$ will be used to
1011 quantify variability for this uncertainty component. This component is evaluated as a
1012 rectangular distribution:
1013



1014
1015
1016 *Upper limit = +a*
1017 *Lower limit = -a*
1018 *Possible range of values = (+a) - (-a) = 2a*
1019

1020 As explained above, for a rectangular distribution, the standard uncertainty is calculated
1021 by:

1022
1023
$$\text{Standard uncertainty} = \frac{a}{\sqrt{3}}$$

1024
1025 The standard uncertainty for the pipette diluter in this example is based on the outside limit
1026 of 3%:
1027

1028
$$\text{Standard uncertainty} = \frac{3\%}{\sqrt{3}} = 1.7321\%$$

1029
1030 **Step 5 Calculate the combined standard uncertainty**

1031
1032 The evaluation will assume that the uncertainty components are independent or
1033 uncorrelated and that the measurement result is the sum of a series of components.
1034

1035 Care shall be taken if the measurement results lie over a range of values. In this scenario,
1036 the calibration model was determined during method validation and was shown through
1037 the use of residual plots to have constant variance across the linear range, so a single
1038 estimation of measurement uncertainty can be calculated for the entire concentration
1039 range.
1040

1041
$$u_c(y) = \sqrt{s_{\text{reproducibility}}^2 + u_{\text{matrix}}^2 + u_{\text{CRMunc}}^2 + u_{\text{pipettediluter}}^2}$$

1042

1043
1044

$$u_c(y) = \sqrt{2.4101^2_{reproducibility} + 2.8868^2_{matrix} + 1.165^2_{CRMunc} + 1.7321^2_{pipettediluter}}$$

1045
1046

$$u_c(y) = \sqrt{18.4992}$$

1047
1048

$$u_c(y) = 4.3011\%$$

1049 **Evaluation of bias**

1050

1051 The laboratory views the monitoring of bias as a component of ensuring the validity of the
1052 test method and has incorporated three CRMs at a low, medium and high concentration as
1053 QC samples for the purpose of monitoring bias from unidentified sources on an ongoing
1054 basis.

1055

1056 The laboratory procedure requires each measured value for a CRM to be within 5 % of the
1057 reference value. The largest bias for any of the control levels (low, medium, and high) is
1058 less than the combined standard uncertainty. Although the bias is viewed as insignificant,
1059 the laboratory is choosing to include an additional component in the uncertainty
1060 evaluation that will address the uncertainty in the reference value of the CRM used for the
1061 evaluation of bias. Steps 3, 4, and 5 must be addressed for this additional uncertainty
1062 component.

1063

1064 **Step 3 Quantify uncertainty components - bias component**

1065

1066 The laboratory reviewed all of the calibration certificates from all CRMs used for the
1067 evaluation of bias. The greatest uncertainty is 0.0014 % for the 0.3 % CRM.

1068

1069

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

1070

1071 **Step 4 Convert quantities to standard uncertainties - bias component**

1072

1073 The certificate indicates this expanded uncertainty assumes a normal distribution, a
1074 coverage factor of $k = 2$ and a coverage probability of approximately 95 %. The
1075 uncertainty on the calibration certificate will be divided by the coverage factor, 2, to
1076 arrive at a standard uncertainty.

1077

1078

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

1079

1080 **Step 5 Calculate combined standard uncertainty - including bias component**

1081

1082 The revised RSS calculation:

1083

1084
1085

$$u_c(y) = \sqrt{s_{reproducibility}^2 + u_{matrix}^2 + u_{CRMunc}^2 + u_{pipettediluter}^2 + u_{CRMbias}^2}$$

1086
1087

$$u_c(y) = \sqrt{2.4101^2_{reproducibility} + 2.8868^2_{matrix} + 1.165^2_{CRMunc} + 1.7321^2_{pipettediluter} + 0.2334^2_{CRMbias}}$$

1088
1089

$$u_c(y) = \sqrt{18.5536}$$

1090
1091

$$u_c(y) = 4.3074 \%$$

1092
1093

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

1094
1095
1096
1097

The data from the measurement process is assumed to follow a normal distribution. The laboratory has 101 measurements of the blood matrix quality control sample. Therefore, the laboratory assumes a lower bound on the effective degrees of freedom for the combined standard uncertainty of 100.

1098
1099
1100

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 will be used.

1101
1102
1103

$$U = 2.025 \times 4.3074 = 8.7225 \%$$

1104
1105

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

1106
1107

Step 7 Evaluate the expanded uncertainty

1108
1109

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

1110
1111

- Stakeholder interests

1112

Expanded uncertainty (8.7225 %) was below a requirement of 10 %.

1113

- Legal requirements

1114

There were none.

1115

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

1116

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

1117

- Established criteria including control limits for method

1119

The laboratory's control acceptance limits for the method are 5 % or 0.005 g/dL,

1120

whichever is larger. Considering the expanded uncertainty, the allowable control

1121

limits were modified to 8 % or 0.008 whichever is larger to minimize the occurrence of excessive QC failures.

1122

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1127 **Step 8 Report the uncertainty**

1128

1129 The laboratory has established a procedure for rounding the expanded uncertainty.

1130 Following that procedure, the expanded uncertainty was rounded to two significant
1131 figures:

1132

1133

$$U = 8.7 \%$$

1134

1135 For reporting measurement results with the rounded expanded uncertainty to the same
1136 number of decimal places:

1137

1138 *“The concentration of ethanol in Item 1 was found to be 0.090 g/dL ± 0.008 g/dL at a*
1139 *coverage probability of 95.45%.”*

1140

DRAFT

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ANNEX B
(Informative)

Concentration of amphetamine and methamphetamine in a whole blood specimen¹¹

Test Method Information

The laboratory developed and validated a test method for quantitation of amphetamine and methamphetamine in whole blood, using liquid chromatography – tandem mass spectrometry (LC-MSMS). Multiple analysts were trained and qualified to use the laboratory’s procedure. All analysts 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 5 concentrations from 10 to 1000 ng/mL. The calibrators are prepared from a working stock solution that was 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 quadratic regression model. Changing variance across the concentration range (heteroscedasticity) was observed across the concentration range.

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

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

Measurement Traceability

The traceability for this measurement process is established through the calibrators used to generate the calibration curve on the measuring system, as well as 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 Practices for Measurement Traceability in Forensic Toxicology*.

All external calibrations of measuring equipment are performed by calibration laboratories that meet the *ANSI/ASB Standard 017, Standard Practices for Measurement Traceability in Forensic Toxicology*. The pipettes and volumetric flasks have been and are routinely calibrated.

¹¹ An evaluation of measurement uncertainty is specific to the measurement traceability that has been established for the measurement, the measurement assurance processes that are in place, the laboratory test method, the laboratory facility, etc. Therefore, the example that follows shall be evaluated and revised by each laboratory to take into consideration the elements that are specific to that laboratory.

1181
1182 **Measurement Assurance**
1183
1184 The QC samples at low (30 ng/mL), medium (400 ng/mL), and high (800 ng/mL)
1185 concentrations are fortified into whole blood from a working stock solution by the
1186 laboratory with each batch. The working stock solution for the controls are prepared from
1187 CRMs purchased from a different supplier than the CRMs used as calibrators. The QC
1188 samples are used to ensure validity of the test method across the concentration range and
1189 to evaluate the method's bias on an ongoing basis.

1190
1191 The laboratory has 15 measurements made of the QC samples during validation for each
1192 concentration.

1193
1194 Since two analytes are involved in this measurement procedure, two separate uncertainty
1195 evaluations will be needed.

1196
1197 **Step 1 Specify the measurement process**

1198
1199 The measurement processes can be described in a written statement:

1200
1201 *"The Concentration of Amphetamine in Whole Blood using [the validated laboratory*
1202 *procedure]"*

1203
1204 *"The Concentration of Methamphetamine in Whole Blood using [the validated*
1205 *laboratory procedure]"*

1206
1207 **Step 2 Identify uncertainty components**

1208
1209 The following list of *possible* contributors to uncertainty in this method were identified by
1210 the laboratory:

1211
1212 Analyst

- 1213 ● Inter-analyst variation in sample preparation and measurements
1214 ● Training
1215 ● Experience

1216
1217 Calibrators Preparation

- 1218 ● Components:
1219 ● Methanol – reagent grade
1220 ● Concentration – equipment used to prepare (pipettes, volumetric flask)
1221 ● CRMs – uncertainty in the stated reference value

1222
1223 Quality Controls Preparation

- 1224 ● Components:
1225 ● Methanol – reagent grade

- 1226 ● Concentration – equipment used to prepare (pipettes, volumetric flask)
- 1227 ● CRMs – uncertainty in the stated reference value

1228

1229 Internal Standard Preparation

- 1230 ● Components:
 - 1231 ● Methanol – reagent grade
 - 1232 ● Stable isotope labeled amphetamine and methamphetamine
- 1233 ● Impurities in the internal standard (unlabeled drug)
- 1234 ● Concentration – equipment used to prepare (pipettes, volumetric flask)

1235

1236 Preparation of aliquots of Calibrators, Quality Control Samples and Measurand

- 1237 ● Homogenization
 - 1238 ● Test Specimens – mixing
- 1239
- 1240 ● Temperature
 - 1241 ● All calibrators, quality control samples and the test specimens are brought to room temperature
 - 1242
 - 1243 ● Variation in the time allowed to reach room temperature
 - 1244 ● Variation in room temperature at different times of year
- 1245
- 1246 ● Pipettes
 - 1247 ● Volume of sample, calibrators, controls and internal standard
 - 1248 ● Calibration uncertainty or laboratory specification to verify calibration status

1249

1250 Analysis

- 1251 ● Instrument parameter settings (e.g., gradient, flow rate, aging of chromatographic column, autosampler syringe, autosampler precision, etc.)
- 1252
- 1253 ● Interference from the matrix
- 1254 ● Interference from reagents
- 1255 ● Interference from other compounds
- 1256 ● Stability of sample(s) from preparation through analysis
- 1257 ● Instrument precision
- 1258 ● Systematic instrumental variation within an analytical batch
- 1259 ● Matrix effect (ionization suppression/enhancement)

1260

1261 Data Processing

- 1262 ● Calibration model
- 1263 ● Integration parameters
- 1264 ● Processing algorithms

1265

1266 NOTE: This list of uncertainty components to be considered could also be compiled into a
1267 fishbone diagram or into any other format of the laboratory's choosing.

1268

1269 NOTE: A laboratory may identify different uncertainty components when an evaluation of
1270 their specific measurement process is performed.

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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 through the use of residual plots to have some heteroscedasticity (the variance was not constant across the linear range). Therefore, the laboratory is going to 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 analysts have contributed to the 15 replicate measurements of the quality control samples at each concentration.

Table 2 shows the individual uncertainty components and how they will be evaluated:

Table 2: Method of Evaluation of Uncertainty Components (Example 2)

Uncertainty Component	Method of Evaluation
Analysts	
Inter-analyst variation	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Training	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Experience	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Calibrators Preparation	
Components: Methanol – reagent grade	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Concentration CRM – uncertainty in the stated reference value Equipment used to prepare (pipettes, volumetric flask)	<i>Type B Evaluation</i>
Quality Control Samples Preparation	
Components: Methanol – reagent grade	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Concentration CRM – uncertainty in the stated reference value Equipment used to prepare (pipettes, volumetric flask)	<i>Type B Evaluation (if necessary for bias)</i>
Internal Standard Preparation	

Components: Methanol – reagent grade	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Stable isotope labeled amphetamine and methamphetamine Impurities in the internal standard (unlabeled drug)	No influence Certificate of analysis from 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
Preparation of aliquots of Calibrators, Quality Control Samples and Test Specimens	
Homogenization – mixing	Demonstrated during method validation to be insignificant.
Temperature – all calibrators, quality 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 <i>Type A Evaluation</i> of process reproducibility data
Pipettes: Volume of sample, calibrators, quality controls, and internal standard Calibration uncertainty or laboratory specification to verify calibration status	Volume of internal standard adequately represented by the <i>Type A Evaluation</i> of process reproducibility data <i>Type B Evaluation</i> 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 <i>Type A Evaluation</i> of process reproducibility data
Interference from the matrix	Matrix interference was evaluated during method validation and found to be insignificant for the matrix type allowed in this method.
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 that does evaluate all reagents used in the analytical method. The laboratory procedure specifies acceptable criteria for this quality 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 <i>Type A Evaluation</i> of process reproducibility data
Instrument precision	Adequately represented by the <i>Type A Evaluation</i> 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 <i>Type A Evaluation</i> of process reproducibility data
Integration parameters	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data
Processing algorithms	Adequately represented by the <i>Type A Evaluation</i> of process reproducibility data

1290

1291 ***Type A Evaluation of uncertainty components***

1292

1293 **Measurement Process Reproducibility**

1294

1295 The number of observations of each QC sample is 15. The statistic that will be calculated is the
1296 percent relative standard deviation.

1297

1298 Through validation, it was determined that the variance was not consistent across the calibration
1299 range. Therefore the reproducibility data from the multiple QC sample levels for either target
1300 compound may not be combined. The 400 ng/mL QC sample had the greatest variance and will be
1301 used for this evaluation.

1302

1303 To begin, the mean (average) and standard deviation of the control data will be calculated.

1304

1305 • The mean of the reproducibility data in this example is 404 ng/mL for amphetamine and 416
1306 ng/mL for methamphetamine.

1307

1308 • The standard deviation of the reproducibility data in this example is 15.90 ng/mL for
1309 amphetamine and 12.01 ng/mL for methamphetamine.

1310

1311 The %RSD of the reproducibility data in this example is 3.936 % for amphetamine and 2.888 %
1312 for methamphetamine.

1313

1314 ***Type B Evaluation of uncertainty components***

1315

1316 **Calibrators Preparation**

1317

1318 ***Uncertainty in the reference value***

1319

1320 The laboratory reviewed the calibration certificates from all CRMs used for the preparation of the
1321 calibration working stock solutions. The largest uncertainty was 0.005 mg/mL for the 1.000 mg/mL
1322 amphetamine CRM and 0.006 mg/mL for the 1.000 mg/mL methamphetamine CRM.

1323

1324
$$\text{Relative uncertainty of Amphetamine CRM} = \left(\frac{0.005 \text{ mg/mL}}{1.000 \text{ mg/mL}} \right) * 100 = 0.5 \%$$

1325

1326
$$\text{Relative uncertainty of Methamphetamine CRM} = \left(\frac{0.006 \text{ mg/mL}}{1.000 \text{ mg/mL}} \right) * 100 = 0.6 \%$$

1327

1328 **Uncertainty in pipettes**

1329

1330 The laboratory reviewed the calibration certificates of all pipettes that may be used for preparation
1331 of the calibration working stock solution. The largest uncertainty was 0.74 μL for a 100 μL pipette.

1332

1333
$$\text{Relative uncertainty of Pipettes to Prep Cal Working Stock} = \left(\frac{0.74 \mu\text{L}}{100 \mu\text{L}} \right) * 100 = 0.74 \%$$

1334

1335 **Uncertainty in volumetric flasks**

1336

1337 The laboratory reviewed the calibration certificates of all volumetric flasks that may be used for
1338 preparation of the calibration working stock solution. The largest uncertainty was 0.0086 mL for a
1339 25mL volumetric flask.

1340

1341
$$\text{Relative uncertainty of Vol Flask to Prep Cal Working Stock} = \left(\frac{0.0086 \text{ mL}}{25 \text{ mL}} \right) * 100 = 0.0344 \%$$

1342

1343 **Preparation of aliquots of Calibrators and Test Specimens**

1344

1345 **Uncertainty in pipettes**

1346

1347 The laboratory reviewed the calibration certificates of all pipettes that may be used to fortify the
1348 calibrators from the working stock solution into whole blood. The method requires the same
1349 pipette to be used to add the internal standard to calibrators, controls, and test specimens. The
1350 largest uncertainty was 0.74 μL for a 100- μL pipette.

1351

1352
$$\text{Relative uncertainty of Pipettes to Fortify Calibrator Samples} = \left(\frac{0.74 \mu\text{L}}{100 \mu\text{L}} \right) * 100 = 0.754 \%$$

1353

1354
$$\text{Relative uncertainty of Pipettes to Delivery Internal Standard} = \left(\frac{0.74 \mu\text{L}}{100 \mu\text{L}} \right) * 100 = 0.74 \%$$

1355

1356 The laboratory reviewed the calibration certificates of all pipettes that may be used to aliquot the
1357 test item. The largest uncertainty was 6.9 μL for a 1000- μL pipette.

1358

1359
$$\text{Relative uncertainty of Pipettes to Aliquot Test Samples} = \left(\frac{6.9 \mu\text{L}}{1000 \mu\text{L}} \right) * 100 = 0.69 \%$$

1360

1361 **Step 4 Convert quantities to standard uncertainties**

1362

1363 **The measurement unit**

1364

1365 In this example the estimated relative uncertainty is expressed as a percentage.

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Type A Evaluation of uncertainty components

Measurement Process Reproducibility data

The % RSD (s_r) of the reproducibility data in this example is 3.936 % for amphetamine and 2.888 % for methamphetamine.

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 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.

$$\text{Relative standard uncertainty of Amphetamine CRM} = \left(\frac{0.5\%}{2}\right) = 0.25\% = u_{CRM}$$

$$\text{Relative standard uncertainty of Methamphetamine CRM} = \left(\frac{0.6\%}{2}\right) = 0.30\% = u_{CRM}$$

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- μ L pipette.

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 relative uncertainty derived from the calibration certificate will be divided by the coverage factor, 2.87, to arrive at a relative standard uncertainty.

$$\text{Relative standard uncertainty of Pipettes to Prep Calib Working Stock} = \left(\frac{0.74\%}{2.87}\right) = 0.258\% = u_{CRMp}$$

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 25mL flask.

The volumetric flask's calibration certificate indicates 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.

1413

1414 *Relative standard uncertainty of Vol Flasks to Prep Calib Working Stock* = $\left(\frac{0.0344\%}{2}\right) = 0.172\% = u_{CRMv}$

1415

1416 **Preparation of aliquots of Calibrators and Test Specimens**

1417

1418 ***Uncertainty in pipettes***

1419

1420 In Step 3, the laboratory determined that among the pipettes used to fortify the calibrators from the
 1421 working stock solution into whole blood, the largest relative uncertainty was 0.74 % for a 100µL
 1422 pipette. The same pipette is used to fortify all samples with the internal standards.

1423

1424 The pipette's calibration certificate indicates this expanded uncertainty assumes a normal
 1425 distribution, a coverage factor of $k = 2.87$ and a coverage probability of approximately 95 %. The
 1426 uncertainty derived from the calibration certificate will be divided by the coverage factor to arrive
 1427 at a relative standard uncertainty.

1428

1429 *Relative standard uncertainty of Pipettes to Fortify Calibrator Samples* = $\left(\frac{0.74\%}{2.87}\right) = 0.258\% = u_{CALp}$

1430

1431 *Relative standard uncertainty of Pipette to Deliver Internal Standard* = $\left(\frac{0.74\%}{2.87}\right) = 0.258\% = u_{ISp}$

1432

1433

1434 The laboratory also determined in Step 3 that among the pipettes used to aliquot test specimens,
 1435 the largest relative uncertainty was 0.69 % for a 1000-µL pipette.

1436

1437 The pipette's calibration certificate indicates this expanded uncertainty assumes a normal
 1438 distribution, a coverage factor of $k = 2.87$, and a coverage probability of approximately 95 %. The
 1439 uncertainty on the calibration certificate will be divided by the coverage factor, 2.87, to arrive at a
 1440 relative standard uncertainty.

1441

1442 *Relative standard uncertainty of Pipettes to Aliquot Test Samples* = $\left(\frac{0.69\%}{2.87}\right) = 0.24\% = u_{ITEMp}$

1443

1444 **Step 5 Calculate the combined standard uncertainty**

1445

1446 The evaluation will assume that the uncertainty components are independent or uncorrelated and
 1447 that the measurement result is the sum of a series of components.

1448

1449 For Amphetamine:

1450

1451
$$u_c(y) = \sqrt{3.936_r^2 + 0.25_{CRM}^2 + 0.258_{CRMp}^2 + 0.0172_{CRMv}^2 + 0.258_{CALp}^2 + 0.258_{ISp}^2 + 0.24_{ITEMp}^2}$$

1452

1453
$$u_c(y) = \sqrt{15.8122}$$

1454

1455
$$u_c(y) = 3.9765\%$$

1456

1457

1458 For Methamphetamine:

1459

$$1460 \quad u_c(y) = \sqrt{2.888_r^2 + 0.30_{CRM}^2 + 0.258_{CRMp}^2 + 0.0172_{CRMv}^2 + 0.258_{CALp}^2 + 0.258_{ISp}^2 + 0.24_{ITEMp}^2}$$

1461

$$1462 \quad u_c(y) = \sqrt{8.6881}$$

1463

$$1464 \quad u_c(y) = 2.9476 \%$$

1465

1466 **Evaluation of bias**

1467

1468 The laboratory in this example views the monitoring of bias as a component of ensuring the validity
1469 of the test method and has incorporated three controls prepared from CRMs at a low, medium and
1470 high concentration as QC samples for the purpose of monitoring bias from unidentified sources on
1471 an ongoing basis.

1472

1473 The largest average bias for any of the control levels (low, medium and high) during validation was
1474 -2.4 % for amphetamine and 4.0 % for methamphetamine.

1475

1476 The bias for amphetamine is less than the combined standard uncertainty (3.9765 %) and is
1477 therefore insignificant. No additional component for the uncertainty of the CRM used to evaluate
1478 bias will be added.

1479

1480 The bias for methamphetamine is greater than the combined standard uncertainty (2.9476 %) and
1481 is therefore significant. Steps 3, 4, and 5 must be addressed for the methamphetamine bias
1482 component.

1483

1484 **Step 3 Quantify uncertainty components – bias component**

1485

1486 During validation the largest bias for methamphetamine was quantified to be 4.0 %.

1487

1488

1489 **Step 4 Convert quantities to standard uncertainties – bias component**

1490

1491 The laboratory has chosen option 4.2.6.1.1.1 (c) to address the bias for methamphetamine
1492 that was determined to be significant. Following the guidance in section 3.2.5.5 of NIST SOP
1493 29, the bias is treated as an uncorrected systematic error and the following equation
1494 applying a rectangular distribution is used to address the uncertainty of the difference
1495 component (u_d) in the MU evaluation:

1496

$$1497 \quad u_d = \frac{bias}{\sqrt{3}} = \frac{4.0}{\sqrt{3}} = 2.3094$$

1498

1499

1500

1501

1502

1503

1504 **Step 5 Calculate combined standard uncertainty – including bias component**

1505
1506 For Methamphetamine the updated root sum of the squares:
1507

1508
$$u_c(y) = \sqrt{2.888_r^2 + 0.30_{CRM}^2 + 0.37_{CRMP}^2 + 0.0172_{CRMV}^2 + 0.258_{CALP}^2 + 0.258_{ISP}^2 + 0.24_{TEMP}^2 + 2.3094_d^2}$$

1509
1510
$$u_c(y) = \sqrt{14.0918}$$

1511
1512
$$u_c(y) = 3.7539 \%$$

1513
1514 **Step 6 Expand the combined standard uncertainty by coverage factor (k)**

1515
1516 The data from the measurement process is assumed to follow a normal distribution. The laboratory
1517 has 15 measurements of the 400 ng/mL QC control. Therefore, the laboratory assumes that the
1518 effective degrees of freedom for the combined standard uncertainty cannot be lower than 14.

1519
1520 Refer to the Student's *t*-distribution table to determine the *k* factor.

1521
1522 To expand the uncertainty to a 95.45 % coverage probability for this example the coverage factor *k*
1523 = 2.20 will be used.

1524
1525 For Amphetamine:

1526
1527
$$U = 2.20 \times 3.9765 = 8.4079 \%$$

1528
1529 For Methamphetamine:

1530
1531
$$U = 2.20 \times 3.7539 = 8.2586 \%$$

1532
1533 **Step 7 Evaluate the expanded uncertainty**

1534
1535 The laboratory determined that the evaluation of uncertainty is fit-for-purpose based on the
1536 following considerations:

- 1537
- 1538 ● Stakeholder interests
1539 There were none.
 - 1540 ● Legal requirements
1541 There were none.
 - 1542 ● The relationship between the reported test value and the expanded MU
1543 Expanded uncertainty as a percentage across the analytical range ensures a consistent
1544 relationship.
 - 1545 ● Established criteria including control limits for method
1546 The laboratory's control limits for the method are 20 %. The allowable control limits were
1547 modified to 10 % for amphetamine and for methamphetamine to reflect the expanded
1548 uncertainty.

1549
1550
1551

1552 **Step 8 Report the uncertainty**

1553
1554 The laboratory has established a procedure for the process of rounding the expanded uncertainty.
1555 Following that procedure, the expanded uncertainty rounded to two significant figures:

1556
1557 For Amphetamine:

1558
1559 $U = 8.4\%$

1560
1561 For Methamphetamine:

1562
1563 $U = 8.3 \%$

1564
1565 For reporting measurement results with the rounded expanded uncertainties to the same number
1566 of decimal places:

1567
1568 *"The concentration of amphetamine in Item 1 was found to be 90 ± 8 ng/mL at a coverage*
1569 *probability of 95.45%. The concentration of methamphetamine in Item 1 was found to be 143*
1570 *± 12 ng/mL at a coverage probability of 95.45%."*

1571
1572
1573

ANNEX C

(Informative)

1574

1575

1576

1577 **Calibration of breath alcohol measuring instrumentation using long-term calibration**
1578 **data from a single instrument¹²**

1579

1580 **Calibration Method Information**

1581 The calibration of an individual breath alcohol instrument uses dry gas measurement
1582 standard data from the current calibration as well as historical calibration data for this
1583 single instrument over time. The calibration method uses measurement standards at
1584 multiple concentrations.

1585

1586 The calibration method does require each concentration of the dry gas measurement
1587 standards to be evaluated in triplicate. The method requires each of the triplicate
1588 measurements to be within 3 % or 0.003 g of ethanol/210 L of breath (g/210 L), whichever
1589 is greater, of the certified reference value of the measurement standard. Furthermore, the
1590 method requires that there shall be no greater than 0.003 g/210 L difference in all three
1591 measurements at each concentration.

1592

1593 **Step 1 Specify the measurement process**

1594

1595 Calibration of breath alcohol measuring instrumentation using long-term calibration data
1596 from a single instrument

1597

1598 **Step 2 Identify uncertainty components**

1599

1600 The following list of *possible* contributors to uncertainty in the calibration method were
1601 identified:

1602

1603 Analyst

1604 ● Inter-analyst variation in performing calibration

1605 ● Training

1606 ● Experience

1607

1608 Breath Alcohol Measuring Instrument Being Calibrated

1609 ● Variability of instrument over time

1610

1611 Measurement Standards

¹² An evaluation of measurement uncertainty is specific to the measurement traceability that has been established for the measurement, the measurement assurance processes that are in place, the laboratory calibration method, the laboratory facility, etc. Therefore, the example that follows shall be evaluated and revised by each laboratory to take into consideration the elements that are specific to that laboratory.

- 1612 • Dry Gas Certified Reference Materials - uncertainty in the stated reference value

1613

1614 Environmental Conditions

- 1615 • Barometric pressure
- 1616 • Humidity
- 1617 • Temperature

1618

1619 Varying Facilities/Location Change

- 1620 • Instrument transport
- 1621 • Power fluctuation

1622

1623 Data Processing

- 1624 • Processing algorithms

1625

1626 **Step 3 Quantify uncertainty components**

1627

1628 Measurement standard data has been collected from use of this calibration method over
 1629 time. All analysts have participated in acquiring the measurement standard data for this
 1630 single breath alcohol measuring instrument. The laboratory has 51 measurements made
 1631 using each measurement standard. The instrument has demonstrated constant variance
 1632 across the concentration range of the measurement standards used in the calibration
 1633 method. Because the 0.100 g/210 L measurement standard has the greatest observed
 1634 variance of the measurement standards, it will be used to represent the process
 1635 reproducibility data.

1636

1637 Table 1 shows the individual uncertainty components and how they will be evaluated:

1638

1639

Table 1: Method of Evaluation of Uncertainty Components (Example 3)

1640

Uncertainty Component	Method of Evaluation
Analysts	
Inter-analyst variation	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Training	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Experience	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Breath Alcohol Measuring Instrument Being Calibrated	
Variability of instrument over time	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Measurement Standards	

CRM –uncertainty in the stated reference value	<i>Type B Evaluation</i>
Environmental Conditions	
Barometric pressure	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Humidity	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Temperature	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard
Varying Facilities/Locations	
Instrument transport	Not Applicable
Power fluctuations	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard.
Data Processing	
Processing algorithms	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – measurement standard

1641

1642 ***Type A Evaluation of uncertainty components***

1643

1644 **Measurement Standard Reproducibility – 0.100 g/210 L Measurement Standard**

1645

1646 The number of observations in this example is 51. The statistic that will be calculated is the
1647 standard deviation.

1648

1649 To begin, the mean (average) and standard deviation of the measurement data will be
1650 calculated.¹³

1651

1652 The mean is calculated as:

1653

1654

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

1655

1656

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

¹³ 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.

1657

1658 The mean of the reproducibility data in this example = 0.0994 g/210 L

1659 The standard deviation is calculated as:

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

1660

1661 The standard deviation of the reproducibility data in this example = 0.0012 g/210 L

1662 **Type B Evaluation of uncertainty components**

1663

1664 **Certified Reference Materials**

1665

1666 Based on the certificates from the CRMs, the laboratory determined in Step 3 that the
1667 greatest relative uncertainty for the CRM was 0.0018 g/210 L for the 0.100 g/210 L CRM.

1668 The certificate indicates this expanded uncertainty assumes a normal distribution, a
1669 coverage factor of $k = 2$ and a coverage probability of approximately 95 %. The uncertainty
1670 on the calibration certificate will be divided by the coverage factor to arrive at a relative
1671 standard uncertainty.

1672

1673
$$\text{Relative standard uncertainty} = \left(\frac{0.0018 \text{ g / 210L}}{2} \right) = 0.0009 \frac{\text{g}}{210} \text{L}$$

1674

1675 **Step 4 Convert quantities to standard uncertainties**

1676

1677 **The measurement unit:** g of ethanol/210 L of breath (g/210 L)

1678

1679 **Type A Evaluation of uncertainty components**

1680

1681 **Measurement Standard Reproducibility – 0.100 g/210 L Measurement Standard**

1682

1683 The standard deviation of the reproducibility data in this example is 0.0012 g/210 L.

1684

- 1685 • No additional conversion is necessary to reach a standard uncertainty.

1686

1687 **Type B Evaluation of uncertainty components:**

1688

1689 **Certified Reference Materials**

1690

1691 The CRM certificate indicates that the stated expanded uncertainty assumes a normal
1692 distribution, a coverage factor of $k = 2$ and a coverage probability of approximately 95 %.

- 1693 • The uncertainty is stated to be 0.0018 g/210 L for the 0.100 g/210 L CRM.
- 1694 • The uncertainty on the calibration certificate will be divided by the coverage factor,
- 1695 2, to arrive at a standard uncertainty.
- 1696 • $0.0018 \text{ g/210 L} / 2 = 0.0009 \text{ g/210 L}$ for the standard uncertainty

1697
1698 **Step 5 Calculate combined standard uncertainty**

1699
1700 The evaluation will assume that the uncertainty components are independent or
1701 uncorrelated and that the measurement result is the sum of a series of components. The
1702 combined standard uncertainty was calculated.

1703
$$u_c(y) = \sqrt{s_{reproducibility}^2 + u_{CRMunc}^2}$$

1704
1705
$$u_c(y) = \sqrt{0.0012_{reproducibility}^2 + 0.0009_{CRMunc}^2}$$

1706
1707
$$u_c(y) = \sqrt{0.0012_{reproducibility}^2 + 0.0009_{CRMunc}^2}$$

1708
1709
$$u_c(y) = \sqrt{2.25 \times 10^{-6}}$$

1710
1711
$$u_c(y) = 0.0015 \text{ g/210L}$$

1712
1713 **Evaluation of Bias**

1714
1715 In this example, bias is evaluated as part of instrument calibration.

1716 The data for the 0.100 g/210 L measurement standard shows a difference of the average to
1717 reference value of 0.001 g/210 L. This value is less than the combined standard uncertainty
1718 and therefore, is insignificant. No additional component will be added to the measurement
1719 uncertainty evaluation.

1720 **Step 6 Expand the combined standard uncertainty by coverage factor (k)**

1721
1722 The laboratory has 51 measurements of the measurement standard. Therefore, the
1723 laboratory assumes a lower bound on the effective degrees of freedom for the combined
1724 standard uncertainty of 50.

1725
1726 The data from the measurement process is assumed to follow a normal distribution;
1727 therefore, refer to the Student's *t*-distribution table to determine the *k* factor.

1728 To expand the uncertainty to a 95.45 % coverage probability for this example the coverage
1729 factor *k* = 2.05 (n=50) will be used.

1730 A laboratory can choose to increase the coverage probability.

1731 $k = 2.05$

1732 $U = 2.05 \times 0.0015 = 0.00308 \text{ g}/210\text{L}$

1733 **Step 7 Evaluate the expanded uncertainty**

1734

1735 The calibration laboratory determined that the evaluation of uncertainty is fit-for-purpose.

1736

1737 The laboratory identified that the current method allows for a variance of 0.005 g/ 210L or
1738 5%, whichever is greater, from a measurement standard known reference value. However,
1739 this is greater than the expanded uncertainty at 95.45 %. Left unchanged, a calibration
1740 could be reported that would have a bias that is significant. Therefore, the laboratory
1741 revised the method so that the variability allowed in any calibration must be equal to or
1742 less than the 0.003 g/ 210L or 3% whichever is greater.

1743

1744 **Step 8 Report the uncertainty**

1745

1746 The laboratory has established a procedure for the process of rounding the expanded
1747 uncertainty. Following that procedure, the expanded uncertainty is rounded to the third
1748 decimal place to equal the number of decimal places reported in the breath alcohol
1749 instrument display. The expanded uncertainty will be 0.003 g/210 L.

1750

1751 The certificate of calibration shall contain:

1752

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

1753

- $k = 2.05$, the coverage factor based on the student's t distribution.

1754

- 95.45 %, the coverage probability

1755

1756

1757 *For reporting calibration results use the rounded expanded uncertainty to the same level of*

1758 *significance*

1759

1760 $0.100 \text{ g}/210 \text{ L} \pm 0.003 \text{ g}/210 \text{ L}$ at a coverage probability of 95.45 % ($k=2.05$)."

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ANNEX D
(Informative)

Calibration of breath alcohol measuring instruments using control data from the calibration method¹⁴

Calibration Method Information

A population of breath alcohol measuring instruments is calibrated using the same calibration method. The calibration method includes multiple measurement standards of varying concentrations and a control. The control data obtained is from a population of 100 breath alcohol measuring instruments that have all demonstrated constant variance across the measurement standard concentration levels. Three measurements of the 0.100 g of ethanol/210 L of breath (g/210 L) control is made during each instrument calibration. The current calibration as well as 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 were identified:

Analyst

- Inter-analyst 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

Calibration Method Control

¹⁴ An evaluation of measurement uncertainty is specific to the measurement traceability that has been established for the measurement, the measurement assurance processes that are in place, the laboratory calibration method, the laboratory facility, etc. Therefore, the example that follows shall be evaluated and revised by each laboratory to take into consideration the elements that are specific to that laboratory.

- 1799 ● Dry Gas Certified Reference Material from a different manufacturer than that of the
1800 Measurement Standards - uncertainty in the stated reference value

1801

1802 Environmental Conditions

- 1803 ● Barometric pressure
1804 ● Humidity
1805 ● Temperature

1806

1807 Varying Facilities/Location Change

- 1808 ● Instrument transport
1809 ● Power fluctuations

1810

1811 Data Processing

- 1812 ● Processing algorithms

1813

1814

1815 **Step 3 Quantify uncertainty components**

1816

1817 The calibration laboratory has existing data from the calibration method. Each instrument
1818 is evaluated, in triplicate, using a 0.100 g/210 L dry gas cylinder with measurement
1819 traceability as a calibration control. The calibration method requires the control to be
1820 within 3 % or 0.003 g/210 L (whichever is greater) of the certified reference value.
1821 Furthermore, there shall be no greater than 0.003 g/210 L difference in all three
1822 calibration control values.

1823

1824 Control data is collected on an on-going basis with all analysts contributing to the control
1825 data for the population of instruments.

1826

1827 Table 1 shows the individual uncertainty components and how they will be evaluated:

1828

1829

Table 1: Method of Evaluation of Uncertainty Components (Example 4)

Uncertainty Component	Method of Evaluation
Analysts	
Inter-analyst variation	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Training	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Experience	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Breath Alcohol Measuring Instrument Being Calibrated	
Population of 100 breath alcohol measuring instruments	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control

Variability of instrument over time	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Measurement Standards	
CRM –uncertainty in the stated reference value	<i>Type B Evaluation</i>
Calibration Method Control	
CRM –uncertainty in the stated reference value	<i>Type B Evaluation</i>
Environmental Conditions	
Barometric pressure	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Humidity	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Temperature	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control
Varying Facilities/Locations	
Instrument transport	Not Applicable
Power fluctuations	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control.
Data Processing	
Processing algorithms	Adequately represented by <i>Type A Evaluation</i> of process reproducibility data – control

1830

1831 **Type A Evaluation of uncertainty components**

1832

1833 **Calibration Control Reproducibility – 0.100 g/210 L Calibration Control**

1834

1835 The number of measurements of the control in this example is greater than 300.

1836

1837 The statistic that will be calculated is the standard deviation.

1838

1839 To begin, the mean (average) and standard deviation of the measurement data will be calculated.¹⁵

1840

1841

1842

Mean

1843

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

1844

¹⁵ 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.

1845
$$\bar{x} = \frac{(x_1 + x_2 + x_3 + \dots x_n)}{n}$$

1846

1847 The mean of the reproducibility data in this example = 0.0996 g/210 L

1848 Standard Deviation

1849
$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

1850 The standard deviation of the reproducibility data in this example = 0.0012 g/210 L

1851

1852 **Type B Evaluation of uncertainty components**

1853

1854 **Certified Reference Materials**

1855

1856 The calibration laboratory reviewed the certificates of analysis from all dry gas cylinders.

1857 The greatest uncertainty is 0.0018 g/210 L for the 0.100 g/210 L CRM.

1858

1859 **Step 4 Convert quantities to standard uncertainties**

1860

1861 **The measurement unit:** g of ethanol/210 L of breath (g/210 L)

1862

1863 **Type A Evaluation of uncertainty components**

1864

1865 **Calibration Control Reproducibility - 0.100 g/210 L Calibration Control**

1866

1867 The standard deviation of the reproducibility data in this example is 0.0012 g/210 L.

- 1868 ● No additional conversion is necessary to reach a standard uncertainty.

1869

1870 **Type B Evaluation of uncertainty components**

1871

1872 **Certified Reference Materials**

1873

1874 The certificates of analysis state that the expanded uncertainty assumes a normal
1875 distribution, a coverage factor of $k = 2$ and a coverage probability of approximately 95 %.

- 1876 ● The greatest uncertainty is 0.0018 g/210 L.

- 1877 ● The uncertainty on the calibration certificate will be divided by the coverage factor,
1878 2, to arrive at a standard uncertainty.

- 1879 ● $0.0018 \text{ g/210 L} / 2 = 0.0009 \text{ g/210 L}$ for the standard uncertainty.

1880

1881

1882 **Step 5 Calculate combined standard uncertainty**

1883
 1884 The evaluation will assume that the uncertainty components are independent or
 1885 uncorrelated and that the measurement result is the sum of a series of components. The
 1886 combined standard uncertainty was calculated.

$$u_c(y) = \sqrt{s_{reproducibility}^2 + u_{CRMunc}^2}$$

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

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

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

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

1897 **Evaluation of Bias**

1898
 1899 In this example, bias is evaluated as part of the instrument calibration. The calibration
 1900 method requires the control to be within 3 % or 0.003 g/210 L (whichever is greater) of
 1901 the certified reference value. Furthermore, there shall be no greater than 0.003 g/210 L
 1902 difference in all three calibration control values.

1903 The data for the 0.100 g/210 L calibration control shows a difference between the average
 1904 and the reference value of 0.001 g/210 L. This value is less than the combined standard
 1905 uncertainty and therefore, is insignificant. Although the bias is viewed as insignificant, the
 1906 laboratory is choosing to include an additional component in the uncertainty evaluation. An
 1907 uncertainty contributor equal to the uncertainty of the reference value of the calibration
 1908 control used for the bias evaluation was added to the evaluation of measurement
 1909 uncertainty.

1910 **Step 3 Quantify uncertainty components – bias component**

1911
 1912 The laboratory noted the difference of the average data for the 0.100 g/210 L
 1913 calibration to be 0.001 g/210 L.

1914
 1915 **Step 4 Convert quantities to standard uncertainties – bias component**

1916
 1917 The standard uncertainty for the bias was 0.001 g/210 L.

1918
 1919 **Step 5 Calculate combined standard uncertainty – including bias component**

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The updated RSS calculation:

$$u_c(y) = \sqrt{s_{reproducibility}^2 + u_{CRMunc}^2 + u_{bias}^2}$$

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

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

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

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 laboratory has 300 measurements of the calibration control. Refer to the Student’s t-distribution table to determine the k factor.

To expand the uncertainty to a 95.45 % coverage probability for this example the coverage factor k = 2.0 will be used.

A laboratory can choose to increase the coverage probability.

$$k = 2.0$$

$$U = 2.0 \times 0.0018 = 0.0036 \text{ g/210L}$$

Step 7 Evaluate the expanded uncertainty

The calibration laboratory determined that the evaluation of uncertainty is fit-for-purpose.

Step 8 Report the uncertainty

The laboratory has established a procedure for the process of rounding the expanded uncertainty. Following that procedure, the expanded uncertainty rounded to the third decimal place. The expanded uncertainty will be 0.004 g/210 L.

The certificate of calibration shall contain:

- 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

1958 *For reporting calibration results use the rounded expanded uncertainty to the same level of*
1959 *significance*

1960

1961 *0.100 g/210 L ± 0.004 g/210 L at a coverage probability of 95.45 % (k=2.0)."*

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ANNEX E
(informative)
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