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Standard for Breath Alcohol Measuring Instrument Calibration

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Foreword

The field of Toxicology includes Breath Alcoholbreath alcohol testing. Breath Alcoholalcohol testing is widely used to determine the alcohol (ethanol) content of an individual. Breath Alcohol Programs vary widely in their requirements (statutory, regulatory, programmatic), resources, and oversight/administration. Historically, the National Safety Council (Alcohol, Drugs and Impairment Division, previously known as Committee on Alcohol and Other Drugs) has outlined initial minimum guidelines for various components of Breath Alcohol testing[1]. breath alcohol testing. This document provides a model for Breath Alcohol Programs to follow in developing and validating a calibration method. Additional program components will be included in other documents.

This document was prepared and finalized as a standard by the Toxicology Consensus Body of the ASB. The draft was developed by the Toxicology Subcommittee of the Organization of Scientific Area Committees for Forensic Science to provide minimum standards of practice for the calibration method development, the validation of evidentiary Breath Alcoholsuch a method and the calibration of breath alcohol instruments. This document provides a model used for Breath Alcohol programs to follow in developing and validating a calibration method.forensic purposes. By following these standards, a Breath Alcohol programProgram will be able to objectively show that a Breath Alcoholbreath alcohol instrument is capable of successfully performing at its intended level of accuracy and precision using the validated calibration method.

The American Academy of Forensic Sciences established the Academy Standards Board (ASB) in 2016 with a vision of safeguarding Justice, Integrity and Fairness through Consensus Based American National Standards. To that end, the ASB develops consensus based forensic standards within a framework accredited by the American National Standards Institute (ANSI), and provides training to support those standards. ASB values integrity, scientific rigor, openness, due process, collaboration, excellence, diversity and inclusion. ASB is dedicated to developing and making freely accessible the highest quality documentary forensic science consensus Standards, Guidelines, Best Practice Recommendations, and Technical Reports in a wide range of forensic science disciplines as a service to forensic practitioners and the legal system.

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

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

ASB procedures are publicly available, free of cost, at www.asbstandardsboard.org.

Keywords: *Breath Alcoholbreath alcohol, calibration, methodology, validation.*

[1] National Safety Council, *A HISTORY of THE COMMITTEE ON ALCOHOL AND OTHER DRUGS (CAOD),* http://www.nsc.org/NSCDocuments_Advocacy/NSChistoryofCAOD.pdf, accessed 4/21/2017.

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Standard for Breath Alcohol Measuring Instrument Calibration

1 Scope

This standard is applicable to the calibration of breath alcohol measuring instruments for evidentiary purposes. These minimum requirements are included for (1) the development and validation of calibration methods on these instruments; (2) evaluation of performance following adjustments and calibrations; and (3) monitoring the validity of the calibrations performed. This standard is not intended to cover preliminary (non-evidentiary) testing, ignition interlock, or federally-regulated testing.

2 Normative References

~~There are no normative reference documents. Annex B, Bibliography, contains informative references.~~

For dated references, only the cited edition applies. For undated references, the latest edition including amendments applies.

ANSI/ASB Standard 017, *Standard Practices for Measurement Traceability in Forensic Toxicology*.[b]

3 Terms and Definitions

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

3.1
adjustment
A set of operations carried out on a measuring system so that it provides prescribed indications corresponding to given values of the quantity to be measured.[c] ~~.~~

3.2
~~**bias**~~
~~Difference~~
bias
An estimate of systematic measurement error, calculated as the difference between the mean of several measurements under identical conditions, to a known "true" value. It is often reported as a percent difference.

3.3
Breath Alcohol Program
An organizational structure including policies, procedures, responsibilities and resources necessary for implementing core ~~Breath Alcohol~~ breath alcohol activities. The Breath Alcohol Program includes, but may not be limited to, requirements or specifications for reference ~~materials~~material,

[b] Available from: https://asb.aafs.org/wp-content/uploads/2018/06/017_Std_e1.pdf
[c] Joint Committee for Guides in Metrology (JCGM), *International vocabulary of metrology - Basic and general concepts and associated terms (VIM)* (Sèvres, France: International Bureau of Weights and Measures [BIPM]-JCGM 200) available from: https://www.bipm.org/en/publications/guides~~).~~

training of operators, maintenance and calibration of instrumentation, the evidential ~~Breath Alcohol~~breath alcohol test sequence, and record retention.

3.4
calibration
Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an ~~indication[2].~~indication.[1]

3.5
calibrator
A reference standard or reference material of known concentration used to standardize or calibrate an instrument or laboratory procedure.

~~3.5~~3.6
carryover
~~Appearance~~The appearance of unintended analyte signal in samples after the analysis of a positive sample.

~~**1.1**~~
~~**interferences**~~
~~Non-targeted analytes (e.g., matrix components, other drugs and metabolites, impurities) which may impact the ability to detect, identify, or quantitate a targeted analyte.~~

3.7
computer system
A system containing one or more components and elements such as computers (hardware), associated software, and data (e.g., software, firmware, hardware, configuration files). [d]

3.8
data
A quantitative or qualitative representation that is observed, measured, collected, or gathered that characterizes some static or dynamic attribute of the physical world or the use of it by individuals or groups of people and that is suitable for communication, interpretation, or processing by humans or machines.[e]

~~3.6~~3.9
Lower Limit of Quantitation
LLOQ
~~The~~ An estimate of the lowest concentration of ~~a measurand~~an analyte in a sample that can be reliably measured ~~by an analytical procedure.~~with acceptable bias and precision.

[d] ISO/IEC 25024:2015(en) *Systems and software engineering — Systems and software Quality Requirements and Evaluation (SQuaRE) — Measurement of data quality* available from: www.webstore.ansi.org.
[e] ASTM E867-06 (2020) *Standard Terminology Relating to Vehicle Pavement Systems* available from: www.webstore.ansi.org.

~~3.7~~3.10
masking
Automated function where results above or below a ~~pre-~~specified threshold are reported ~~as no analyte (e.g., with~~ a defined result. For example, the instrument may report "ethanol ~~not present, below administrative threshold~~" or "0.000 g/210 L ethanol~~).~~" for a response below 0.005 g/210 L. Another example may be reporting "Results greater than 0.400 g/210 L" or "Over Range" for a response above 0.400 g/210 L.

3.11
measured quantity value
A quantity value representing a measurement result.[f]

~~3.8~~3.12
measurement assurance
The process of monitoring the validity of the calibrations performed.

3.13
nominal quantity value
A rounded or approximate value of a characterizing quantity of a measuring instrument or measuring system that provides guidance for its appropriate use.[5]

~~3.9~~3.14
precision
The measure of the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogenous sample. It is expressed numerically as the coefficient of variation (% CV).

~~3.10~~3.15
reference material
~~Material~~A material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.[g]~~.~~

~~3.11~~3.16
reporting range
~~Range~~A range of concentrations that can be reliably measured by an analytical procedure.

~~3.12~~3.17
stability
An analyte's resistance to chemical change in a matrix under specific conditions for given time intervals.

[f] Joint Committee for Guides in Metrology (JCGM), *International vocabulary of metrology - Basic and general concepts and associated terms (VIM)* (Sèvres, France: International Bureau of Weights and Measures [BIPM]- JCGM 200) available at https://www.bipm.org/en/publications/guides.

[g] Joint Committee for Guides in Metrology (JCGM), *International vocabulary of metrology - Basic and general concepts and associated terms (VIM)* (Sèvres, France: International Bureau of Weights and Measures [BIPM]- JCGM 200~~)~~ available at https://www.bipm.org/en/publications/guides~~).~~

~~3.13~~3.18
Upper Limit of Quantitation
ULOQ
The highest concentration of an analyte in a ~~measurand~~sample that can be reliably measured ~~by an analytical procedure~~with acceptable bias and precision.

4 ~~The~~Development of a Calibration Method ~~(Development and Optimization)~~

~~1.2 General~~

4.1 The calibration method shall be a defined procedure with specified components and pre-defined acceptance criteria. Breath Alcohol Programs (hereafter called Program) providing calibration services for evidentiary ~~Breath Alcohol~~breath alcohol instruments are often subject to legal, programmatic, legal precedent, and/or accreditation requirements. Consequently, the Program may need to perform various experiments to develop and optimize a method that meets Program requirements~~.~~ and the requirements of this standard.

4.2 Prior to performing calibration method validation experiments, all ~~components~~elements of the calibration method shall be determined and defined. The Program may choose to revise computer system parameters during the method development phase to optimize components such as method, instrumentation, or user interface.

4.3 Accuracy (bias and precision) is integral to calibration methods. The needs of the end user should be balanced against instrument capabilities. Bias and precision results obtained during method optimization should be used to determine the method's acceptance criteria.

4.4 The largest calculated within-run and between-run % CV for each concentration shall be used to determine precision acceptability.

4.5 During method development, the LLOQ and ULOQ shall be determined. The range of ethanol concentrations of interest (e.g., statutory concentrations, administrative concentrations) shall be considered when determining the appropriate proposed limits.

4.5.1 The following is one of multiple paths that can be used to determine these values.

a) Determine ULOQ using reference material with established traceability.

b) Analyze a minimum of three ethanol concentrations around the proposed upper limit .

c) Each concentration should be analyzed three consecutive times (replicates).

d) The concentrations should bracket the proposed upper limit (e.g., 0.38, 0.400, 0.420 for a proposed 0.400 ULOQ).

e) The highest data point where acceptable bias and precision criteria are met is the experimentally determined ULOQ.

f) If the series of 3 concentrations does not meet the bias and precision criteria, a series of 3 lower concentrations shall be repeated until acceptable bias and precision is achieved.

4.5.2 Determine LLOQ using reference material with established traceability.

a) Analyze a minimum of three ethanol concentrations around the proposed lower limit .

b) Each concentration should be analyzed three consecutive times (replicates).

c) The concentrations should bracket the proposed lower limit (e.g., 0.015, 0.020, 0.025 for a proposed 0.020 LLOQ).

d) The lowest data point where acceptable bias and precision criteria are met is the experimentally determined LLOQ.

e) If the series of 3 concentrations does not meet the bias and precision criteria, a series of 3 higher concentrations shall be repeated until acceptable bias and precision is achieved.

4.6 If masking is to be utilized during testing, this function shall be removed during performance of the calibration method. The specific concentrations at which point masking occurs shall be determined during the method development and optimization phase.

4.7 The usage, storage, and transportation requirements for reference material may need to be modified to eliminate limitations. In cases when it is not possible to modify, limitations shall be documented in the calibration method.

4.24.8 The final calibration method, however determined, shall be validated prior to use on instruments for evidential purposes. Annex BA provides an example of a method development and optimization plan with example results.

5 Elements of a Calibration Method

4.35.1 The calibration method shall include, but may not be limited to, the following.:

a) Methodmethod name.;

b) Instrumentinstrument make and model.;

NOTE This document does not address instrument specifications; however, the instrument make and model shall be specified in the calibration method.

c) Computercomputer system parameters.:

1) Analysisanalysis and the subsequent information obtained (e.g., diagnostics, response curves), calculated (e.g., results), retained, and reported is controlled by a computer system. .

2) Componentscomponents of the computer system (e.g., software, firmware, hardware, configuration files)parameters which shall be uniquely identified and versioned if applicable.;

1) The Program may choose to revise computer system parameters during the method development phase to optimize components such as method, instrumentation, or user interface. However, the computer system parameters shall remain the same throughout validation and subsequent evidentiary testing.

d) ~~Reference~~reference material~~.~~:

1) ~~Matrix: ~~matrix:

i) aqueous (wet), and/or

ii) compressed (dry) gas;

~~1) source (traceability, uncertainty);~~

~~1)~~2) concentrations;

~~2)~~3) ~~number of different~~the reporting range is dependent upon the calibrator concentrations;

~~i. minimum of 4 non-zero calibrators if the measurement technology (detector) is inherently linear;~~

— The lowest reported concentration shall be equal to the lowest non-zero calibrator utilized in the calibration method.

— The highest reported concentration shall be equal to the highest calibrator utilized in the calibration method.

~~3)~~4) a minimum of 6 non-zero concentrations shall be used as calibrators ~~if measurement technology is not linear~~;

5) the concentrations shall span the calibration range;

~~4)~~6) number of replicates per concentration ~~(~~shall be a minimum of 5~~).~~

e) ~~Limits~~limits of quantitation (See ~~section 4.~~4~~.3.3).~~);

f) ~~Reporting~~reporting range~~.~~:

— The calibration method shall define the reporting range. The calibration method shall ensure acceptable results across the entire reporting range. The reporting range may be administratively set but shall be within the validated reporting range. Legally mandated ethanol concentrations should guide the decision regarding the reporting range.

g) ~~Calibration~~calibration sequence~~.~~:

— The calibration sequence is comprised of the number of replicates, number of concentrations, and the order of operations performed during the calibration method. Programs may use an automated process for their calibration sequence.

h) ~~Acceptance~~traceability;

— Traceability shall be established according to ANSI/ASB Standard 017, *Standard Practices for Measurement Traceability in Forensic Toxicology*.

i) process to evaluate the calibration method's measurement uncertainty;

h)j) acceptance criteria.;

— Criteria shall be defined for a successful calibration. The method shall also specify steps to be taken when the calibration does not meet the parameters for successful calibration.

6 ValidatingValidation of a Calibration Method

6.1 When to Validate the Calibration Method

~~1.2.1 When to validate the calibration method.~~

6.1.1 Calibration methods shall be validated when it is necessary to verify a method's performance parameters are acceptable for use. ~~Common examples requiring validation include:~~

~~a)~~ Common examples requiring validation include the following:

a) existing calibration ~~methods~~method that ~~do~~does not currently meet the current requirements outlined in this document;

b) ~~b)~~ modifications of an established calibration method to improve performance or extend its use beyond that for which it was originally validated (e.g., expanded reporting range);

c) ~~c)~~ new calibration method;

d) ~~d)~~ to demonstrate equivalent uniformity between an established method/instrument and a new method/instrument.;

e) and modification to the computer system parameter(s). Not all modifications impact the calibration method. The Program shall perform acceptance testing on revised system parameter(s) to determine impact (if any) on the calibration method.

6.1.2 The parameters to be evaluated for validation of calibration methods ~~will~~ depend upon the circumstances in which the method is to be used. Likewise, it is recognized that after validation has occurred, methods may be revised. The extent and frequency of revalidation of previously validated methods ~~will~~ depend upon the nature of the intended changes or Program policy. See Section ~~4.4~~7 for further ~~guidance~~information on revalidation of ~~previously validated~~ methods.

6.1.3 A Program using a method that was validated prior to the promulgation of this document shall demonstrate and document that the method meets the Program's needs (i.e., fit-for-purpose) under this standard. The method is likely to have sufficient historical calibration data that can be used to address a number of the required validation parameters. In the absence of sufficient data to fulfill these minimum standards, appropriate studies shall be conducted to ensure conformance with this document.

6.2 Establishing a Validation Plan

6.2.1 The Program is responsible for ensuring the calibration method is satisfactorily validated. Annex B provides examples of a calibration method validation plan.

6.2.2 A validation plan shall be in place prior to starting any validation experiments.

NOTE The validation plan is typically separate from a ~~Program's~~Programs standard operating procedure (SOP) for method validation ~~and it~~. It provides direction for the specific experiments that will be performed and acceptance criteria for each parameter.

6.2.3 The plan shall specify that the method (including computer system parameters) shall remain the same throughout validation.

6.2.4 The plan shall include the ~~elements~~parameters specified in Section 4.~~4. Further, it~~

6.2.5 The plan shall specify acceptance criteria for each parameter necessary to approve the calibration method.

~~6.2.1~~6.2.6 The plan shall document the validation and method requirements that ~~will~~may allow it to be ~~acceptable~~ fit ~~for use (e.g., purpose. For example, a Program may require~~ the calibration ~~shall be accurate in temperatures from~~method to appropriately perform at a specific temperature range of –5°C to 40°C~~). The plan shall also define the role(s) and responsibility(ties) of all personnel involved in the validation. Annexes C and D provide examples of a calibration method validation plan and selected results.~~(in a non-controlled environment).

6.2.7 The ~~Program~~plan shall ~~determine~~specify the number of instruments to be used for validation experiments. A minimum of 1 instrument shall have all validation experiments performed in totality.

NOTE Although a minimum of 1 instrument is specified for method validation, all instruments undergo performance verification and calibration prior to evidential use.

~~6.2.2~~6.2.8 The validation shall be conducted using the same calibration conditions and parameters as specified in the final method. ~~A minimum of 1 instrument shall have all validation experiments performed in totality[h].~~

6.2.9 The validation plan shall require successful completion of all validation experiments on the same method prior to approval.

~~6.2.3~~6.2.10 Programs should consider uncertainty estimation in developing the validation plan. ~~Validation experiment data may be used to calculate the initial estimation of measurement uncertainty.~~

~~6.3~~ Validation Parameters

~~6.3.1~~6.2.11 General

~~6.3.2~~6.2.12 ~~It is important to evaluate all applicable validation parameters and address them in the validation plan. Alternatively, parameters may be addressed through other means (e.g., quality assurance practices, published references) and documented within the validation plan.~~

~~[h] Although a minimum of only 1 instrument is specified for method validation, all instruments shall undergo performance verification and calibration prior to evidential use~~

All validation experiments outlined below shall be conducted in similar environments and conditions in which a calibration may take place. Validation experiments shall be conducted on different days ~~and by~~. Programs should use different analysts ~~(i.e., other than whomever calibrated the instrument, if~~when practicable~~).~~.

~~6.3.3~~6.2.13 Bias and Precision

~~6.3.3.1~~6.2.13.1 General

~~Bias~~Accuracy (bias and precision) shall be ~~calculated for each instrument (if performing validation experiments using more than one instrument)~~measured using reference material with established traceability ~~at five separate~~that is different than that used for the calibration. A minimum of three concentrations ~~across the reporting range[i]. Each concentration~~ (low, medium, high) shall be ~~run~~evaluated over six different days, with a minimum of ~~five consecutive times (~~three replicates~~).~~ of each concentration per day.

~~Programs that utilize masking during testing may need to cancel masking during validation to determine bias and precision at lower concentrations.~~

Low concentrations shall be no more than approximately 3 times the lowest calibrator utilized in the calibration method and high concentrations shall be no less than 80% of the highest calibrator utilized in the calibration method. Medium concentrations shall be near the midpoint of the low and high concentrations.

When using compressed gas reference material, the results shall be normalized for standard atmospheric pressure before bias and precision evaluation. See Annex C for example calculations.

~~6.3.3.2~~6.2.13.2 Bias Determination and Acceptance

The bias shall be calculated for each concentration. See Annex D for example validation data. The Program may utilize the nominal quantity value or ~~known~~measured quantity value of the reference material to calculate the bias, however, it shall be specified in the validation plan. See Annex C for guidance related to nominal quantity and measured quantity values.

For calculating bias utilizing the nominal quantity value, use the following formula:

$$\textbf{\textit{Bias}}\ (\%)\ \textbf{\textit{at Concentration}}_x = \left[\frac{\text{Grand Mean of Calculated Concentration}_x - \text{Nominal Concentration}_x}{\text{Nominal Concentration}_x}\right] \times 100 \qquad (1)$$

For calculating bias utilizing the ~~target~~measured quantity value, use the following formula:

$$\textbf{\textit{Bias}}\ (\%)\ \textbf{\textit{at Concentration}}_x = \left[\frac{\text{Grand Mean of Calculated Concentration}_x - \text{Known Concentration}_x}{\text{Known Concentration}_x}\right] \times \left[\frac{\text{Grand Mean of Calculated Concentration}_x - \text{Measured Concentration}_x}{\text{Measured Concentration}_x}\right] \times 100 \qquad (2)$$

~~[i] For purposes of this document, low concentrations shall be no more than approximately 3 times the lowest end of the reporting range of the method and high concentrations shall be within approximately 80% (or more) of the highest end of the reporting range of the method. Medium concentrations shall be near the midpoint of the low and high concentrations.~~

The maximum acceptable bias is ±5% or 0.005 g/210 L, (whichever is greater) at each concentration.

~~6.3.3.3~~6.2.13.3 Precision

Precision is expressed as the coefficient of variation (% CV). The mean and standard deviation (~~SD~~std dev) of the ~~replicates. The following formula~~response is ~~used~~calculated for each concentration to ~~calculate~~determine the ~~standard deviation:~~% CV.

~~$$SD = \sqrt{\frac{\sum(x_i - m)^2}{n-1}} \quad (3)$$~~

~~where $\sum$ means "sum of", x_i is a value in the data set, m is the mean of the data set,~~

$$\%\ CV = \frac{std\ dev}{mean\ response} \times 100 \quad (3)$$

The % CV shall not exceed ± 10%. The largest calculated within-run and ~~n is the number of data points.~~

~~The maximum acceptable standard deviation is less than or equal to 1/3 of the maximum acceptable bias~~between-run % CV for each concentration~~.~~ not exceed ± 10%.

6.2.13.4 Within-Run Precision Calculations

Within-run precisions are calculated for each concentration separately for each of the six runs. Within-run precision may be calculated using the data from each run's triplicate analyses at each concentration as:

$$Within - Run\ \%\ CV = \frac{std\ dev\ of\ a\ single\ run\ of\ samples}{mean\ calculated\ value\ of\ a\ single\ run\ of\ samples} \times 100 \quad (4)$$

6.2.13.5Between-Run Precision Calculations

Between-run precision is calculated for each concentration over the six runs (minimum n=18/concentration). This may be done by using the combined data from all replicates of each concentration as:

$$Between - Run\ \%\ CV = \frac{std\ dev\ of\ all\ observations\ for\ each\ concentration}{grand\ mean\ for\ each\ concentration} \times 100 \quad (5)$$

~~6.3.4~~6.2.14 Limits of Quantitation

~~4.4.1.1 Determining the LLOQ/~~The ULOQ ~~using reference material with established traceability:~~

~~6.3.4.1~~6.2.14.1 ~~Minimum of three decreasing/increasing ethanol concentrations~~and LLOQ shall be ~~analyzed five consecutive times (replicates);~~determined within the method development process.

~~4.4.1.1.1 The lowest/highest statutorily mandated ethanol level should be considered when determining the appropriate ethanol concentrations to use;~~

~~4.4.1.1.2 The lowest/highest concentration capable of achieving acceptable bias and precision criteria in all three samples is considered the estimated LLOQ/ULOQ.~~

~~6.3.4.2~~6.2.14.2 ~~Alternatively, programs~~Programs may administratively set a ~~LLOQ/ULOQ based upon results gained during development, regulatory, or statutory constraints~~ULOQ and LLOQ; however, the Program shall demonstrate that the ULOQ and LLOQ~~/ULOQ~~ levels chosen, meet acceptable bias and precision criteria.

~~6.3.5~~6.2.15 Carryover

Carryover shall be evaluated as part of method validation. ~~Ethanol~~To evaluate carryover as part of method validation, ethanol negative sample(s) (e.g., human breath, ~~dry~~compressed gas, and/or aqueous solution) shall be analyzed immediately after the highest concentration of the reporting range. ~~This shall be tested using three replicate analyses.~~

~~If possible,~~The highest calibrator concentration at which no ethanol carryover is observed (above the ~~calibration~~method's LLOD) in the ethanol negative sample is determined to be the concentration at which the method ~~will eliminate~~is free from carryover. Determining the concentration at which no carryover exists shall be confirmed by repeating the determination twice (i.e., a total of 3 repeated tests).

The calibration method (or computer parameters) should be modified to remove any carryover. In cases when it is not possible to eliminate the carryover, the ~~calibration method~~ Program shall address in writing how carryover ~~will~~shall be managed.

~~6.3.6~~6.2.16 Reference Material ~~Stability~~

6.2.16.1 Reference material used to calibrate the instrument(s) may be subject to variables including storage, repeated usage, and transportation conditions and handling. The ~~stability~~performance of reference material shall be evaluated ~~as applicable. Stability experiments~~with respect to the calibration method.

~~6.3.6.1~~6.2.16.2 Experiments shall be designed and conducted to address situations typically encountered with reference material used to calibrate a ~~Breath Alcohol~~breath alcohol instrument. Annex E and Annex F provide examples of validation plans related to reference material ~~stability.~~used in the calibration method. Characteristics that ~~may~~shall be evaluated include~~:~~.

~~a) shelf life of reference material;~~

a) ~~stability~~Reference material shall conform to requirements published in ANSI/ASB Standard 017, *Standard Practices for Measurement Traceability in Forensic Toxicology*.

b) Shelf life of reference material if it may be used past the stated date of best use (different terms may be used by manufacturers such as recommended retest date, reanalysis date, and expiration date).

~~a)~~c) Stability of reference material over time and/or usage~~;~~ as permitted in the calibration method. Examples of possible calibration method or Program choices.

1) ~~stability~~The calibration method allows for repeated use of a single aqueous simulator solution over X calendar days or X replicates.

2) The Program allows for a reference material to be repeatedly opened to produce simulator solution which can impact the concentration of ethanol.

~~b)~~d) Stability of reference material when used, stored~~/~~, and/or transported outside of ~~room temperature;~~manufacturer's recommended conditions (see Annex E for an example validation plan).

~~c)~~e) ~~stability~~Capability of compressed gas cylinder at lower pressure ~~(i.~~when used in the calibration method (e~~, amount of~~.g., valve limitations, compressed gas ~~introduced to instrument at lower~~ cylinder ~~pressure).~~performance). See Annex F for an example validation plan.

6.2.16.3 Any limitations related to reference material identified during the validation experiments shall be addressed.

~~6.3.7~~6.2.17 Environmental Conditions

~~6.3.7.1~~**6.2.17.1** The performance of the calibration method shall be assessed under similar environmental conditions that are typically encountered in the laboratory and/or field (as applicable). If environmental conditions exist that potentially cause an effect on the instrument operation and calibration method, then those conditions shall be evaluated. The Program shall define these conditions in the validation plan. The Program shall calibrate the instrument(s) under the defined conditions and then assess the applicable validation parameters. Annex G provides examples for validation plans for environmental conditions. Environmental conditions may include, but are not limited to:

a) atmospheric pressure;

b) humidity;

c) radiofrequency interference (RFI);

d) temperature.

~~6.3.7.2~~**6.2.17.2** Alternatively, parameters may be addressed through other means (e.g., quality assurance practices, published references) and documented within the validation plan.

7 Revalidation of Previously Validated Methods

7.1 Modifications to a validated method require evaluation to confirm that the changes do not have an adverse effect on the method's performance. The decision regarding which performance characteristics require additional validation is based on logical consideration of the specific parameters likely to be affected by the change(s). These changes may include, but are not limited to the following:

a) analytical changes to ~~software/firmware~~the computer system;

b) expanded reporting range;

c) instrumentation (e.g., different model); and

d) location of calibration method performance (e.g., initiating field calibrations).

7.2 For example, an analytical change in ~~software/firmware~~the computer system may affect linearity, ~~physiological influences,~~ precision, or bias. Consideration should be given to conducting parallel studies using a previously validated method and the modified method to evaluate the effects of the change(s). The goal is to demonstrate any impact the change(s) have on the performance of the previously validated procedure. New models/manufacturers of instrumentation shall require a complete validation study. ~~Acquisition of the same model may require limited validation.~~

7.3 ~~Programs~~Laboratories using ~~calibration~~ methods that were validated prior to the ~~publication~~promulgation of this ~~standard~~document shall demonstrate and document that those ~~previous calibration~~ methods are ~~acceptable~~ fit for ~~use~~ purpose under this standard. ~~This calibration method will~~ These methods are likely to have sufficient historical calibration ~~and control~~ data that can be used to address a number of the required validation parameters. ~~Without~~ In the absence of sufficient data to fulfill ~~this~~these minimum ~~standard~~standards, appropriate studies shall be conducted to ensure ~~compliance~~conformance with this document.

8 Validation Documentation Requirements

8.1 Record keeping is an essential part of a ~~Program's~~Programs operating procedures and is a key component of method validation. The following validation records shall be retained, organized, and available for review:

~~a) conclusion/Summary;~~

~~b) date of approval;~~

a) name ~~and title of person(s) approving~~ of the calibration method;

b) validation plan;

~~c) raw data or reference to where the raw data are stored;~~

c) original observations, data and calculations shall be recorded at the time they are made ; observations, data and calculations shall include at a minimum:

1) date, identity of personnel ~~involved in the method validation;~~and breath alcohol instrument(s),

~~1) dates when each parameter was evaluated;~~

2) source of reference material (e.g., lot number, manufacturer~~);~~, dates), and

~~2) limits of quantitation data (see Section 4.4.3).~~

~~d) references;~~

~~e) results and calculations;~~

~~b)~~a) ~~validation plan;~~

~~1) scope;~~

~~2) name of calibration method;~~

3) description of all the parameters evaluated~~.~~, results and calculations. If any of the parameters were not evaluated, then the reason shall be ~~stated or~~documented and justified;

d) references;

e) conclusion/summary;

f) date of approval;

~~c)~~g) and the name and title of person(s) approving the calibration method for use.

8.2 Records shall be retained according to the Program's record retention policy or for a minimum of 10 years after the calibration method is no longer used.

9 Adjustment

9.1 An evidential ~~Breath Alcohol~~breath alcohol instrument may ~~require adjustment~~be adjusted for ~~corrective or~~various reasons (e.g., out of tolerance results, mandated occurrences, preventive reasons~~, or to comply~~).

9.2 Adjustment shall be conducted using reference material with ~~administratively~~ established ~~intervals. Where possible, a~~ traceability that is different than that used for the calibration.

9.3 Instrument performance shall be ~~performed~~evaluated before ~~and after~~ an adjustment~~.~~. Instrument performance shall be evaluated using reference material with established traceability. Reference material used for the calibration may also be used for the performance evaluation.

9.4 To evaluate instrument performance, a minimum of three concentrations (low, medium, high) shall be evaluated. Low concentrations shall be no more than approximately 3 times the lowest calibrator utilized in the calibration method and high concentrations shall be no less than 80% of the highest calibrator utilized in the calibration method. Medium concentrations shall be near the midpoint of the low and high concentrations. Alternatively, the Program may perform their calibration method prior to adjustment to ~~establish the as-found and as-left condition.~~ evaluate instrument performance.

9.5 When using compressed gas reference material, the results shall be normalized for standard atmospheric pressure before bias evaluation.

9.6 If the performance evaluation results do not meet the acceptable bias criteria specified in the calibration method, prior instrument results should be evaluated.

9.7 The results of adjustment(s) shall be documented.

~~9.1~~9.8 An adjustment shall be followed by a calibration before the instrument is used for evidential Breath Alcohol testing. ~~Examples of instances when the as-found calibration may not be possible:~~

9.9 There are limited scenarios where the performance evaluation is not possible. For example:

a) The instrument is unable to run tests until repairs are performed~~.~~; or

b) The instrument software is preventing continued testing without first performing an adjustment.

~~4 Calibration (Logistics)~~

9.10 Instances where a performance evaluation is not performed shall be documented and justified.

10 Performance

~~9.2~~10.1 When to Calibrate

The calibration method shall have a specified interval not to exceed 12 months from the date of calibration. Instruments may be calibrated more frequently. Additionally, instruments used for evidential purposes shall be calibrated under the following circumstances:

~~a)~~ after a ~~firmware/software~~ change ~~that affects~~to the ~~measurement process;~~

~~b) before and after an adjustment (see Section 5.6);~~

a) ~~after any *analytical* sampling~~computer system parameter that impacts the analytical results;

~~a)~~b) after any system component~~(s)~~ that impacts an analytical result is replaced or repaired;

c) after an adjustment (see Section 9);

d) when acceptance criteria are not successfully met (e.g., failed calibration); and

~~b)~~e) prior to ~~initial use~~being used the first time for evidential testing~~;~~.

~~c) statutory, regulatory, programmatic requirement(s).~~

~~6.1 Personnel~~

~~Personnel performing calibration activities shall comply with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard for Breath Alcohol Personnel. ()~~

~~9.3~~10.2 Measurement Assurance

Programs shall have a scheduled process for monitoring the validity of their calibration activities. Techniques to evaluate calibration results may include, but are not limited to, the following: participation in an inter-laboratory proficiency program; routine calibration checks on evidential instruments; and calibration verification using reference ~~materials~~material after a calibration has been performed.

~~9.4~~10.3 Unacceptable Calibration Results

The Program shall define the action(s) to be taken when the calibration ~~method does~~results do not meet the defined acceptance parameters. This response may be a subsequent attempt at calibration, troubleshooting, and/or repair.

~~9.5~~10.4 Documentation Requirements

~~All~~Programs shall retain all records ~~that are produced during the~~ related to calibration ~~shall be retained~~, adjustment, and instrument maintenance according to the ~~Program's~~Programs retention schedule. ~~However, the retention time can be no less than 10 years.~~

~~**6.2 Calibration Certificates**~~

~~10~~11 Elements of a Calibration Certificate

~~Calibration certificates~~A calibration certificate (however named) shall be created for calibrations that meet the acceptance criteria.

Certificates shall be written clearly and shall include at a minimum the following:

a) a description of the calibration ~~item~~ instrument (e.g., instrument make/model);

b) an unambiguous identification of the calibration ~~item~~ instrument (e.g., serial number);

c) date of calibration;

~~a) evidence that~~date of issue of the ~~measurements are traceable;~~

~~b) calibration results, with units of measurement;~~

d) ~~the results before and after any adjustment or repair, if available~~Certificate;

e) the calibration interval (e.g., "The calibration of this instrument is valid for 12 months from the date of calibration");

~~c) the condition of the calibration item instrument (i.e., "as found");~~

f) calibration results, with units of measurement and the associated uncertainty of measurement;

~~f)~~g) the name and address of the Program;

~~d) the name and address of the customer (if different than the Program);~~

~~g)~~h) the name and address where the calibration was performed (if different than the Program's address);

~~h)~~i) the name of the calibration method (e.g., title of standard operating procedure);

~~i)~~j) ~~the name, title, and signature or secure electronic equivalent~~the name of the calibration certificate author (e.g., the individual taking responsibility for the calibration certificate); and

~~e)~~ if certificates are multi-page, all pages shall contain the ~~Program's unique identification for~~page number and the ~~calibration~~final page count (e.g., ~~serial number + date, certificate number);~~

~~f) the uncertainty~~1 of ~~measurement;~~

~~j)~~k) ~~title (e.g., Instrument Calibration Certificate, Certification Record~~3).

~~11~~12 Amended Certificates

When modifications to the original calibration certificate are necessary, an amended certificate shall clearly indicate the amendment. If a new certificate is issued, the certificate shall reference the original certificate.

Annex A
(informative)

~~Foundational Principles~~

~~A scientifically valid program is required to ensure that instruments used for evidentiary Breath Alcohol testing produce accurate and reliable results. Program components include personnel, instrument specifications, calibration protocols, subject testing protocols, and quality assurance procedures. This document focuses on calibration; additional program components will be included in other documents. The calibration of Breath Alcohol instrumentation is the cornerstone of a scientifically valid program.~~

~~The term "calibration" is used in various ways in the scientific community. For Breath Alcohol instrumentation, the calibration involves the analysis of specific known standards to determine the relationship between the expected values and the reported values for a given analyte (i.e., ethanol). This should not be confused with an adjustment, where changes are made to the instrument's response in order to match a known value. Anytime an adjustment is performed on an instrument, a calibration is performed to ensure that the instrument was adjusted as expected.~~

~~Calibration methods are validated to ensure methods are acceptable for their intended use. Validation is the process of performing a set of experiments that reliably estimate the efficacy and reliability of an analytical method. The goal of validation is to establish objective evidence that demonstrates a method is capable of successfully performing at the level of its intended use and to identify the method's limitations under normal operating conditions.~~

~~Annex B~~
~~(informative)~~

Example of a Method Development ~~and Optimization~~ Plan with Selected Results[j]

A.1 Method Development and Optimization Plan

~~B~~A.1.1 Objective

Develop and optimize a calibration method for Instrument ABC that provides the:

a) required minimum bias and precision;

b) necessary analytical capabilities;

c) determination of the lower and upper limits of quantitation;

d) determination of the reporting range.

~~B~~A.1.2 Development Protocol

Instrument –	Instrument ABC equipped with Anytown P.D. computer system (software/firmware) version 05.3
Equipment – metrologically traceable to	Company XYZ ~~Simulators (Temperature~~simulators (temperature SI units)
by an laboratory)	External ~~Barometer (~~barometer (metrologically traceable to SI units accredited ISO/IEC 17025 calibration
Standards – N_2)	For ~~Calibration: Compressed~~calibration: compressed gas (EtOH in (metrologically traceable to SI units)
	For verification of the calibration method during method development: ~~Aqueous~~aqueous reference material (EtOH in H_2O) (metrologically traceable to SI units)
Location(s) –	Anytown P.D. Crime Laboratory 1234 Main Street, Anytown, USA
Maximum bias/precision –	Bias = ~~3~~5% or 0.~~003 g/210 L~~005g/210L (whichever is greater)
	Precision = ~~≤1/3 of the acceptable bias for each concentration~~The % CV shall not exceed +/-10%

[j] This is an example of a mock Method Development/Optimization Plan and subsequent results, for illustrative purposes only. Actual *concentrations of interest and instrument capability may vary.*

Concentrations of ~~Interest~~interest – 0.02 g/210 L of breath = underage DUI

0.04 g/210 L of breath = mass transit operators

0.08 g/210 L of breath = rebuttable presumption of DUI

0.15 g/210 L of breath = enhanced penalty for DUI

0.20 g/210 L of breath = enhanced penalty for DUI

Records – The ~~name(s)~~names and ~~date(s)~~dates of those involved with executing this plan will be recorded with the resultant data.

Analytical process – The following process will be repeated, as necessary, to achieve the stated objective.

a) Determine the Lower Limit of Quantitation (LLOQ).

A minimum of three samples of decreasing ethanol concentrations shall be analyzed ~~five~~three consecutive times (replicates). The lowest statutorily mandated ethanol level ~~(0.02 g/210 L of breath)~~ will be considered when determining the appropriate ethanol concentrations. The lowest concentration that is capable of achieving acceptable bias and precision criteria ~~in all three samples~~ is considered the estimated LLOQ.

b) Determine the Upper Limit of Quantitation (ULOQ).

A minimum of three samples of increasing ethanol concentrations shall be analyzed ~~five~~three consecutive times (replicates). The highest statutorily mandated ethanol level ~~(0.20 g/210 L of breath)~~ will be considered when determining the appropriate ethanol concentrations. The highest concentration that is capable of achieving acceptable bias and precision criteria ~~in all three samples~~ is considered the estimated ULOQ.

c) Determine the reporting range.

The reporting range will be determined using data developed from Step a) and Step b) above. ~~The Concentrations of Interest must also be considered~~The concentrations of interest shall also be considered. Once the reporting range has been determined, no quantitative values will be reported above the upper limit of quantitation. A final computer system version will be programmed for reporting; this final software will go through the method validation testing process.

d) Develop appropriate calibration method(s).

Specify the instrument parameters, concentrations, acceptance parameters, and number of replicates used for each calibration method used during the method development phase.

e) Evaluate the data obtained from Step d) above to determine if further optimization is desired.

f) At the conclusion of Steps a) through e), identify the appropriate calibration method that will advance to the validation stage.

g) The ~~name(s), date(s),~~names, dates, instrument parameters, and final data will be retained until the method validation is successfully concluded.

~~B~~A.2 Method Development Results and Summary

~~B~~A.2.1 Determination of the Lower Limit of Quantitation (LLOQ)

~~Five (5~~Three (3) different metrologically traceable aqueous standards of decreasing concentration were evaluated to determine the LLOQ. It was determined by experiment and manufacturer's literature that the instrument has a masking function at 0.005 g/210L. The LLOQ was ~~determined to be~~administratively set at 0.~~010~~020 g/210L.

Table ~~B~~A.1—Determination of LLOQ

Date/initials:		**8/1/~~14~~ ~~TNW~~2014**	**8/1/~~14~~ ~~TNW~~2014**	**8/1/~~14~~ ~~TNW~~2014**	~~**8/1/14 TNW**~~	~~**8/2/14 TNW**~~
		TNW	**TNW**	**TNW**		
Instrument SN		~~0902999~~90299	~~0902999~~90299	~~0902999~~90299	~~090299~~	~~090299~~
Simulator SN	~~XN1480~~	XN1425	XN1480	XN1454	~~XN1493~~	~~XN1493~~
Sim Solution Lot#	~~140301A~~	140728A	140301A	140301B	~~140728B~~	~~140802A~~
Sim Solution Exp.	~~3/1/15~~	7/28/~~15~~2015	3/1/~~15~~2015	7/28/~~15~~2015	~~8/2/15~~	
~~Target~~**Nominal quantity conc (g/~~210L)~~ 210 L)**	~~**0.020**~~	**0.015**	**0.~~01~~002**	**0.~~005~~025**	~~**0.008**~~	
Replicate #1	~~0.020~~	0.014	0.~~01~~002	0.~~000~~026	~~0.012~~	
Replicate #2		0.~~020~~015	0.~~01~~402	0.~~010~~026	~~0.000~~	~~0.010~~
Replicate #3	~~0.019~~	0.014	0.~~010~~019	0.~~000~~025	~~0.008~~	
~~Replicate #4~~	~~0.019~~	~~0.013~~	~~0.010~~		~~0.000~~	~~0.007~~
~~Replicate #5~~	~~0.019~~	~~0.013~~	~~0.010~~		~~0.000~~	~~0.000~~
~~Mean:~~	~~0.019~~	~~0.013~~	~~0.010~~		~~0.000~~	~~0.007~~
~~Number of Analyses:~~	~~5~~	~~5~~	~~5~~		~~5~~	~~5~~
~~Range (Low-High):~~Mean:	~~0.019-0.020~~	~~0.013~~-0.014	0.~~010-0.010~~020	0.~~000-0.000~~026	~~0.000-0.012~~	
~~± 0.003 acceptable bias range~~Bias		-0.~~017-0.023~~001	-0.~~012-0.0180~~003	0.~~007-0.013~~001	~~0.002-0.008~~	~~0.005-0.011~~
~~Standard Deviation:~~std dev		0.~~00000~~00058	0.~~00000~~00058	0.~~00000~~00058	~~0.000~~	~~0.004~~

% CV:	4.0	2.9	2.2	
Acceptable bias and precision	Yes	Yes	Yes	~~No~~ ~~No~~
Final determined LLOQ:	0.020 g/210 L			

~~B~~

A.2.2 Determination of the Upper Limit of Quantitation (ULOQ)

Four (4) different metrologically traceable aqueous standards of increasing concentration were evaluated to determine the ULOQ. ~~It was determined by experiment and manufacturer's literature that the instrument has an upper detection limit of 0.420 g/210L.~~ The ULOQ was determined to be at 0.400 g/~~210L.~~210 L.

Table BA.2—Determination of ULOQ

Date/initials:	8/2/~~14~~ ~~TNW~~2014	8/2/~~14~~ ~~TNW~~2014	8/2/~~14~~ ~~TNW~~2014	8/2/~~14~~ ~~TNW~~2014
	TNW	TNW	TNW	TNW
Instrument SN	~~09029~~90299	~~09029~~90299	~~09029~~90299	~~09029~~90299
Simulator SN	XN1480	XN1425	XN1454	XN1430
Sim Solution Lot#	140301C	140728C	140301D	140728D
Sim Solution Exp.	3/1/~~15~~2015	7/28/~~15~~2015	3/1/~~15~~2015	7/28/~~15~~2015
Target conc (g/210L)	**0.380**	**0.400**	**0.420**	**0.~~425~~430**
Replicate #1	0.375	0.401	0.~~410~~41	Sample Over Range
Replicate #2	0.~~382~~370	0.~~400~~392	Sample Over Range~~0.412~~	Sample Over Range
Replicate #3	0.~~381~~368	0.~~399~~387	~~0.415~~N/A	Sample Over Range
Mean:~~Replicate #4~~	0.~~379~~371	0.~~399~~393	~~0.419~~N/A	~~Sample Over Range~~N/A
~~Replicate #5~~Minimum acceptable bias	0.~~380~~361	0.~~403~~380	0.399~~Sample Over Range~~	~~Sample Over Range~~0.404
Maximum acceptable bias~~Mean:~~	0.~~379~~399	0.~~400~~420	0.~~414~~441	~~Sample Over Range~~0.446
~~Number of Analyses:~~Bias (%)	~~5~~-2.37	~~5~~-1.67	~~5~~N/A	~~5~~N/A
~~Range (Low-High):~~std dev	0.~~375 - 0.382~~0036	0.~~399 - 0.403~~0071	~~0.410 - 0.419~~[a]N/A	[a]N/A
~~± 3% acceptable bias range~~% CV:	1.0.~~368 - 0.391~~	~~0.388 - 0.412~~1.8	~~0.407 - 0.432~~N/A	~~0.412 - 0.437~~N/A
~~Standard Deviation:~~	~~0.002~~	~~0.001~~	~~0.003~~	[a]
Acceptable bias and precision	Yes	Yes	~~Yes~~**No**	**No**
~~[a] Instrument message "Sample Over Range" at concentrations ≥ upper detection limit~~Final determined ULOQ:	0.400 g/210 L			

BA.3 Method Development

Table A.3 summarizes the instrument parameters, concentrations, acceptance parameters, number of replicates and results.

a) Example using Nominal Quantity Value to calculate Bias Determination

Formula #1 located in Section 6.3.2.~~3~~2:

$$\textit{Bias}\ (\%)\ \textit{at Concentration}_x = \left[\frac{\text{Grand Mean of Calculated Concentration}_x - \text{Nominal Concentration}_x}{\text{Nominal Concentration}_x}\right] \times 100 \quad \text{(A.1)}$$

Bias (%) at Concentration$_{80}$ = ((79.45 mg/dL – 80.00 mg/dL) / 80.00 mg/dL) * 100)
Bias (%) at Concentration$_{80}$ = -0.69%

b) Example using Measured Quantity Value to calculate Bias Determination

Formula #2 located in Section 6.3.2.2:

$$\textit{Bias}\ (\%)\ \textit{at Concentration}_x = \left[\frac{\text{Grand Mean of Calculated Concentration}_x - \text{Measured Concentration}_x}{\text{Measured Concentration}_x}\right] \times 100 \quad \text{(A.2)}$$

Bias (%) at Concentration$_{80}$ = ((79.45 mg/dL – 78.53 mg/dL) / 78.53 mg/dL) * 100)
Bias (%) at Concentration$_{80}$ = 1.17%

for Calibration Method A.

A.3.1 Assessment and Comparison of Method A and Method B

No further optimization is necessary as Calibration Method A meets the requirements.

A.3.2 Determination of the Reporting Range

The LLOQ, ULOQ and concentrations of interest were considered in determining the reporting range~~.~~ (for subject testing). The final reported result has 2 significant figures with units = g/210 L of breath. ~~Therefore, the lowest concentration reported will be 0.01 g/210 L of breath. For results <0.01 g/210 L of breath, the instrument will report 0.00 g/210 L of breath. The highest concentration reported will be 0.40 g/210 L of breath. For results >0.40 g/210 L of breath, the instrument will report "Sample Over Range".~~See Table A.5 for additional information.

Table ~~B.3~~A.5—Determination of the Reporting Range

Concentrations of Interest	Concentration (g/210 L)
LLOQ	0.~~01~~02
Lowest conc. of interest	0.02
~~Lower Reporting Limit~~Lowest (subject) reporting limit	0.~~01~~02
ULOQ	0.40
Highest conc. of interest	0.20
~~Higher Reporting Limit~~Highest (subject) reporting limit	0.40
Resulting ~~Reporting Range~~(subject) reporting range	0.~~01~~02-0.40

~~B~~For results <0.02 g/210 L of breath, the instrument will report the actual result when in calibration mode. For subject testing, these results will be reported as 0.00 g/210 L of breath.

For results >0.40 g/210 L of breath, the instrument will report the actual result when in calibration mode. For subject testing, these results will be reported as "Seek Medical Attention".

A.3.3 Summary of Method Development

~~Tables B.4 and B.5 summarize the instrument parameters, concentrations, acceptance parameters, number of replicates and results for each calibration method (Method A and Method B) used during the method development phase.~~

In August 2015, Anytown PD performed method development for the ABC evidential breath alcohol instrument. The quantitation range was found to be 0.02-0.40 g/ 210L of breath. The (subject) reporting range was established to be 0.02-0.40 g/210L of breath for Method A. Method validation experiments will be performed using Calibration Method A to assess the suitability of the method for evidential calibration purposes.

Table B.4A.3—Summary of Calibration Results Using Method A

METHOD A			Calibration			Verify Verification		
Date/initials	8/15/15 NLT	8/15/15 NLT	8/15/15 NLT	8/15/15 NLT	8/15/15 NLT	8/15/15 NLT	8/15/15 NLT	
Instrument SN	90320090320	90320090320	90320090320	09032090320	09032090320	09032090320	09032090320	
CRM matrix	compressed gas (results normalized)	compressed gas (results normalized)	compressed gas (results normalized)	compressed gas (results normalized)	compressed gas (results normalized)	compressed gas (results normalized)	aqueous	
CRM Lot#	AL141202	AL141204	AL141208	AL141015	AL141220	AL141030	140728D	
CRM Exp.	16 Dec	16 Dec	16 Dec	16 Oct	16 Dec	16 Oct	7/28/15	
Target Nominal quantity conc. (g/210L210 L)	**0.02002**	**0.04004**	**0.08008**	**0.15015**	**0.2002**	**0.3004**	**0.1001**	
Replicate #1	0.01902	0.039	0.079	0.148	0.201	0.302389	0.099	
Replicate #2	0.01902	0.039	0.079	0.149	0.202	0.304378	0.099	
Replicate #3	0.020018	0.039	0.079	0.15015	0.202	0.303382	0.099098	
Replicate #4	0.020018	0.04004	0.08008	0.149	0.201	0.30339	0.098097	
Replicate #5	0.02002	0.04004	0.08008	0.15015	0.203	0.303388	0.1001	
Replicate #6	0.019	0.040	0.080	0.151	0.202	0.304	0.100	
Replicate #7	0.020	0.040	0.081	0.150	0.202	0.303	0.100	
Replicate #8	0.019	0.040	0.081	0.149	0.202	0.304	0.099	
Replicate #9	0.020	0.039	0.080	0.150	0.203	0.304	0.099	
Replicate #10	0.020	0.039	0.081	0.150	0.203	0.304	0.099	
Mean:	0.0200192	0.0400394	0.0800794	0.1501492	0.2022018	0.3033854	0.0990986	

	~~Range (Low-High)~~	~~0.019-0.020~~	~~0.039-0.040~~	~~0.079-0.081~~	~~0.148-0.151~~	~~0.201-0.203~~	~~0.302-0.304~~	~~0.098-0.100~~
(± ~~3~~5% or 0.~~003~~005) acceptable bias range	0.~~017~~015-0.~~023~~025	0.~~037~~035-0.~~043~~045	0.~~077~~075-0.~~083~~085	0.~~145~~142-0.~~154~~157	0.~~194~~190-0.~~206~~210	0.~~291~~380-0.~~309~~440	0.~~097~~095-0.~~103~~105
~~Standard Deviation~~Bias (%)	~~0.001~~N/A	~~0.001~~N/A	~~0.001~~N/A	-0.~~0015~~	0.~~0019~~	~~0.001~~-3.7	~~0.001~~-1.4
Bias (g/ 210 L)	-0.0008	-0.0006	-0.0006	N/A	N/A	N/A	N/A
std dev	0.0011	0.0005	0.0005	0.0008	0.0008	0.0052	0.0011
% CV	5.7	1.4	0.7	0.6	0.4	1.3	1.2
Acceptable bias and precision	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Annex B
(informative)

Table B.5—Summary of Results Using Method B

METHOD B	Calibration				Verify
Date/initials:	8/20/15 NLT	8/20/15 NLT	8/20/15 NLT	8/20/15 NLT	8/20/15 NLT
Instrument SN	090320	090320	090320	090320	090320
CRM matrix	gas	gas	gas	gas	aqueous
CRM Lot#	AL141204	AL141208	AL141015	AL141030	140728D
CRM Exp.	16-Dec	16-Dec	16-Oct	16-Oct	7/28/15
Target conc. (g/210L)	**0.040**	**0.080**	**0.150**	**0.300**	**0.100**
Replicate #1	0.039	0.079	0.148	0.302	0.101
Replicate #2	0.039	0.079	0.149	0.304	0.101
Replicate #3	0.039	0.079	0.150	0.303	0.101
Replicate #4	0.040	0.080	0.149	0.303	0.102
Replicate #5	0.040	0.080	0.150	0.303	0.102
Mean:	0.039	0.079	0.149	0.303	0.102
Range (Low-High):	0.039-0.040	0.079-0.081	0.148-0.151	0.302-0.304	0.101-0.102
(± 3% or 0.003) acceptable bias range	0.037-0.043	0.077-0.083	0.145-0.154	0.291-0.309	0.097-0.103
Standard Deviation:	0.001	0.001	0.001	0.001	0.001
Acceptable bias and precision	Yes	Yes	Yes	Yes	Yes

B.3.1 Assessment and Comparison of Method A and Method B

No further optimization is necessary, both Method A and Method B meet the requirements.

Calibration Method B involves fewer concentrations and replicates to achieve equivalent accuracy and precision. Therefore, Method B will advance to the validation stage.

B.3.2 Summary of Method Development

In August, 2016, Anytown PD performed method development for the ABC evidential Breath Alcohol instrument. The reporting range was determined to be 0.01-0.40 g/210L of breath. Two (2) Calibration Methods were developed and tested. Both Calibration methods met the requirements. Method Validation experiments will be performed using Calibration Method B to assess the suitability of the method for evidential calibration purposes.

~~Annex C~~
~~(informative)~~

Example of a Method Validation Plan[11]~~Method Validation Plan~~

~~C.1~~B.1 Introduction

During **Method Development** it was determined that the ~~LOD~~LLOQ for instrument "Model-123" was 0.01 g/210L, the ~~LLOQ was 0.02 g/210L, the~~ ULOQ was 0.~~60~~40 g/210L, **and** the resulting ~~Measurement~~Reporting Range was 0.02 to 0.~~60~~40 g/210L.

Because this calibration method will serve as a quantitative procedure for evidential use, the method validation parameters will be assessed for the desired requirements as listed in Table 1. The assessments shall be performed by multiple analysts and in multiple locations, including field locations. The name of the analyst and the location shall be recorded each time.

~~C.1~~B.2 Equipment, Method and Materials

Instrument –	"Model-123" evidential Breath Alcohol measuring instrument, ~~Infrared~~infrared technology calibrated with Method A1216~~.~~
Method –	Method Validation Procedure for Breath Alcohol Instruments (MVP_001), approved 1/1/2015
Simulator –	Model "XYZ" simulators from Company-A, with certified thermometers from Company-B
Reference material –	Aqueous, certified ethanol-water solutions from Company-C, multiple concentrations
Reference material – concentrations	Gas, certified ~~dry~~compressed gas-ethanol from Company-D, multiple
Water –	Laboratory grade deionized water, made in-house
~~Volatiles –~~	~~Laboratory grade acetone, methanol, isopropanol from Company-E~~

The lot numbers of all reference materials and other reagents shall be recorded as well as the serial numbers of all equipment.

[11] This is an example of a mock Method Validation Plan, for illustrative purposes only.

Table ~~C~~B.1—Validation Plan for Instrument "Model-123" (Method A1216)

Parameter (~~Main text~~ANSI/ASB 055 document reference)	**Pre-Determined Acceptance Criteria**	**~~Assessment~~Validation Parameters**
Bias (see ~~section~~ 6.~~1~~3.2)	Shall not exceed ± 5%, or ± 0.005 g/210L, whichever is greater	10 replicates, ~~5~~6 separate runs, 5 different instruments at the following concentrations: 0.02 g/210L (i.e. statutory limit & LLOQ) ~~0.08 g/210L~~ 10L (statutory limit) 0.15 g/210L (statutory limit) 0.30 g/210L (mid-range concentration) 0.~~60~~40 g/210L (ULOQ) Separately evaluate the bias for each instrument.
Precision (see ~~section~~ 6.~~1~~.3.2)	~~3)~~1) ~~<~~1/3 of the acceptable bias for each concentration	~~Use the same data obtained from the bias study. Separately evaluate the precision for each instrument, for each separate run, by combining the data from all 10 replicates at each concentration (N=10 data points for each concentration).~~Not to exceed +/-10 % CV
~~Endogenous and Physiological Influences~~Carryover (see ~~section~~ 6.3.4)	~~4) Endogenous - No interfering signal from matrix~~ ~~5)~~ ~~6)~~ ~~7)~~2) ~~Physiological - No interfering signal from common volatiles, either alone or in combination with an ethanol reference material solution~~	~~Endogenous (human)~~ ~~10 different sources~~ ~~Part 1, Common Volatiles (5 replicates~~Carryover at ~~low, **target**, high concentrations)~~ ~~Acetone at 0.02 g/210 L, **0.06 g/210L**, 0.10 g/210L~~ ~~Methanol at 0.005 g/210L,~~ULOQ does not exceed 0.01 g/210L~~, 0.03 g/210L~~ ~~Isopropanol at 0.005 g/210L, **0.01 g/210L**, 0.03 g/210L~~ ~~Part 2, Common Volatiles (5 replicates)~~ ~~Repeat Part 1, but with each volatile level spiked in an ethanol solution at 0.10 g/210L~~
~~Carryover (see section 6.2)~~	~~Carryover at ULOQ does not exceed 0.01 g/210L~~	~~For each instrument, follow each 0.60 g/210L calibrator with a blank SIM SOL or air blank, perform in triplicate~~

NOTE Several of the parameters listed in ~~Table B.1~~this table can be assessed simultaneously; for example, the data used for bias assessment can also be used to assess precision

Annex C
(informative)

Calculations (Background and Examples)

Accurate calculations rely on scientific knowledge as well as mathematical skills. This informative annex provides examples for selected calculations. It does not provide the scientific knowledge necessary to ensure the correct formulae are used.

C.1 Bias and Precision

C.1.1 Nominal vs. Measured Quantity Value

Certified Reference Material (CRM) possess a Nominal Quantity Value. This may also be referred to as a 'stated' or 'certified concentration' (e.g., Ethanol-80 has a certified concentration of 80.00 +/- 0.31 mg/dL). In essence, the reference material producer has prepared the reference material to achieve a target concentration (i.e., the target concentration is 80.00 mg/dL in the previous example).

Certified Reference Material should be supplied with a Certificate of Analysis meeting the requirements of ISO 17034. Table C.1 provides an example of a Certificate of Analysis entry with a Nominal Quantity Value of an aqueous ethanol reference material.

Table C.1 – Example of Aqueous Nominal Quantity Value on Certificate of Analysis

Component	Solution Chromatographic Purity	Certified Concentration
Ethanol	> 99.9%	80.00 + 0.31 mg/ dL

The result(s) of the reference material producer's testing process are Measured Quantity Value(s). Measured Quantity Values are frequently included on a Certificate of Analysis (see Table C.2).

Table C.2 – Example of Aqueous Measured Quantity Value on Certificate of Analysis

Solution Standard	Lot Number	Results compared to NIST SRM Lot 1234 (mg/ dL)	Homogeneity (ampoule to ampoule consistency) %RSD
New Lot	ABC123	78.53	1.02%
Prior Lot	ABC124	78.99	1.08%
Acceptance Criteria		± 2%	± 2%

C.1.2 Bias Determination Calculations

Section 6.3.2.2 *Bias Determination and Acceptance* in this document provides two separate bias determination formulae.

To demonstrate the calculations, hypothetical values of a single calibrator are provided below:

Nominal Quantity Value: 80.00 mg/dL Ethanol

Measured Quantity Value: 78.53 mg/dL Ethanol

Grand Mean of validation results: 79.45 mg/dL Ethanol

C.1.3 Within-Run Precision Determination Calculations

a) Table C.3 provides replicate data from a single run.

Table C.3 – Example Data from Calibration Run

Low Calibrator	Concentration (g/210 L)
Rep 1	0.050
Rep 2	0.051
Rep 3	0.048
Mean	0.050
Standard Deviation	0.002

b) Using the data from Table C.3 and the Within-Run Precision formula (#4 located in Section 6.3.2.3) to determine the precision of this calibrator:

$$Within - Run\ CV(\%) = \frac{std\ dev\ of\ a\ single\ run\ of\ samples}{mean\ calculated\ value\ of\ a\ single\ run\ of\ samples} \times 100 \quad \text{(C.1)}$$

$$Within - Run\ \%\ CV = \frac{0.002}{0.050} \times 100$$

$$Within - Run\ \%\ CV = 3.1\%$$

C.1.4 Between-Run Precision Determination Calculations

a) Table C.4 provides validation data for a single concentration (6 separate runs with 3 replicates per concentration).

Table C.4 – Example Data from Validation Experiment

Low Concentration	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	0.050	0.050	0.051	0.049	0.049	0.048
Rep 2	0.049	0.050	0.050	0.048	0.049	0.048
Rep 3	0.049	0.051	0.050	0.049	0.050	0.048
Grand Mean	0.049					
Std Dev	0.001					

b) Using the data from Table C.4 and the Between-Run Precision formula (#5 located in Section 6.3.2.3):

$$Between - Run\ \%\ CV = \frac{std\ dev\ of\ all\ observations\ for\ each\ concentration}{grand\ mean\ for\ each\ concentration} \times 100 \quad \text{(C.2)}$$

$$Between - Run\ \%\ CV = \frac{0.001}{0.049} \times 100$$

$$Within - Run\ \%\ CV = 2.0\%$$

C.2 Converting Aqueous Concentrations to Vapor Concentrations

Breath alcohol measuring instruments that use aqueous reference material measure ethanol concentrations in the vapor phase (headspace). The nominal and measured quantity values published for aqueous reference material arise from the aqueous phase. To accurately use aqueous concentrations in breath alcohol calibrations, a mathematical conversion takes place.

The conversion requires a partition ratio. There are several peer reviewed, published studies outlining aqueous ethanol partition ratios. This informative annex uses the ratio published by A.W. Jones in *Determination of liquid/air partition coefficients for dilute solutions of ethanol in water, whole blood, and plasma*. The water to air partition ratio ($K_{w/a}$) for an aqueous ethanol solution was determined to be 2587 at 34°c. Using this ratio, the following formula can be used to determine the relationship between an aqueous concentration and vapor concentration:

$$Concentration_w = [Concentration_a \times 2587] \div 210 \quad \text{(C.3)}$$

where:

$Concentration_w$ = concentration in the aqueous (water) phase (mg/100 mL)

$Concentration_a$ = concentration in the vapor (air) phase (g/210 L)

2587 = conversion factor [2587 water: 1 air @ 34° c]

210 = conversion factor [g/210 L to mg/100 mL]

Scenario - A Program wants to produce reference material with a targeted 0.080 g/210 L vapor concentration. What aqueous concentration should be prepared? Using the formula above, the calculation becomes:

$Concentration_w = [Concentration_a \times 2587] \div 210$

$Concentration_w = [0.080 \times 2587] \div 210$

$Concentration_w = 0.99$ mg/100 mL

The Program would prepare an aqueous ethanol solution with a target concentration of 0.99 mg/100 mL to achieve a target vapor concentration of 0.080 g/210 L.

C.3 Normalizing a Compressed Gas Result to Standard Atmospheric Pressure

The concentration of compressed gas reference material is impacted by atmospheric pressure. To compare results, they are normalized to standard atmospheric pressure. The following calculation may be used to normalize a result:

Normalized Result (g/ 210 L) = [760/Pressure] × Result (C.3)

where:

Normalized Result (g/210 L) = instrument result normalized to standard atmospheric pressure

Result = the actual measurement result obtained from the instrument (g/210 L)

760 = conversion factor [1 atm = 760 mmHg]

Pressure = barometric pressure at the time of the result (mmHg)

Scenario - An instrument's barometric pressure at 9am MDT was 712.5 mmHg. At 0902 MDT a calibration verification test was performed, with a result of 0.075 g/210 L. What is the normalized result?

Normalized Result (g/ 210 L) = [760/ Pressure] × Result

Normalized Result (g/ 210 L) = [760/ 712.5] × 0.075

Normalized Result (g/ 210 L) = 0.080

The normalized result may now be used to evaluate bias.

C.4 Converting Compressed Gas Results (g/ 210 L to parts per million)

Compressed gas reference material is frequently available with the nominal quantity value (concentration) expressed as both a breath alcohol value (e.g., grams per 210 liters of ethanol vapor) and parts per million (ppm)). The two values are typically present on the Certificate and the cylinder label of CRM. The following formula converts an unknown breath alcohol result (g/210 L to ppm):

$$Result_{ppm} = (Result_{BrAC} \times CRM_{ppm}) \div CRM_{BrAC} \quad (C.4)$$

where:

$Result_{ppm}$ = converted result (ppm)

$Result_{BrAC}$ = instrument (g/210 L)

CRM_{ppm} = certified nominal quality value (ppm)

CRM_{BrAC} = certified nominal quality value (g/210 L)

Scenario - A compressed gas proficiency test cylinder provides a result of 0.150 g/ 210 L ethanol. The label on one of their compressed gas calibrators provides the following information [0.100 g/210 L: 260 ppm]. The proficiency test results are to be recorded in ppm per the proficiency test providers (PTP) instructions. What is the converted result?

$Result_{ppm} = (Result_{BrAC} \times CRM_{ppm}) \div CRM_{BrAC}$

$Result_{ppm} = (0.150 \times 260) \div 0.100$

$Result_{ppm} = 390$

C.5 Corrected and Uncorrected CRM

When compressed gas reference material is run on an electrochemical detector (fuel cell), a small but consistent reduction in expected results occurs. This is addressed by increasing compressed gas concentrations in a systemic manner referred to as the wet/dry offset. This offset may be accomplished by automatically converting results in the software prior to reporting or by increasing the concentration of ethanol in the calibrators. The instrument manufacturer should be consulted to determine the specific wet-dry offset approach utilized.

In instances where CRM with higher concentrations are utilized as calibrators, cylinders may be labelled as Corrected. For example, a manufacturer may specify their instrument has a wet dry offset of 4.5%. A Program may purchase Corrected CRM to calibrate their instruments. The ethanol concentration (in ppm) is increased by 4.5% in the Corrected CRM. The reference material producer (RMP) may label their compressed gas cylinders as Uncorrected vs. Corrected and the ppm will correspond accordingly. Using the above example of a 4.5% wet dry offset, a 0.100 g/210 L nominal quantity value CRM will be labelled as Uncorrected (260 ppm) or Corrected (272 ppm).

C.6 Example of Calculations Used in a Proficiency Test

Scenario - A Program calibrates their instrument using Corrected compressed gas reference material ([0.100 g/210 L:272 ppm]) with a wet-dry offset of 4.5%. The PTP provides four compressed gas cylinders and specifies a ratio of [0.100 g/210 L:260 ppm] ethanol for the proficiency test cylinders. The proficiency test results are to be recorded in g/210 L per the PTP instructions. What steps will the Program take to provide results to the PTP?

Step 1- following calibration, each of the unknowns is run

Table C.5 Proficiency Test Data

-	Concentration (g/210 L)			
Replicate	**PT cylinder 1**	**PT cylinder 2**	**PT cylinder 3**	**PT cylinder 4**
Rep 1	0.042	0.284	0.118	0.069
Rep 2	0.042	0.279	0.120	0.073
Rep 3	0.041	0.280	0.119	0.070
Mean	0.042	0.281	0.119	0.071
Standard Deviation	0.001	0.003	0.001	0.002

Step 2 -for each unknown, calculate the normalized result following Section C.3

Table C.6—Proficiency Test Data Normalized

	PT cylinder 1	PT cylinder 2	PT cylinder 3	PT cylinder 4
Mean	0.042	0.281	0.119	0.071
Standard Deviation	0.001	0.003	0.001	0.002
Barometric Pressure (mmHg)	764.80	764.80	764.80	764.80
Normalized results (g/ 210 L)	0.041	0.279	0.118	0.070

Step 3- convert the normalized results to ppm following Section C.4

Table C.7—Conversion of Breath Alcohol Results (g/210 L to ppm)

	PT cylinder 1	PT cylinder 2	PT cylinder 3	PT cylinder 4
Normalized results (g/ 210 L)	0.041	0.279	0.118	0.070
Normalized results (ppm)	112.62	759.52	321.65	191.01

Step 4- convert the result (ppm) generated from a calibration that utilizes Corrected cylinders to an Uncorrected result (g/210 L)

$$\text{Uncorrected Result (g/210 L)} = (PTP_{g/210\ L} \div PTP_{ppm}) \times \text{Corrected Result}_{ppm} \quad \text{(C.4)}$$

where:

Uncorrected Result (g/210 L) = the breath alcohol result to be reported to the PTP

$PTP_{g/210\ L}$ = part of the conversion factor supplied by the Proficiency Test Provider (0.100g/210 L)

PTP_{ppm} = part of the conversion factor supplied by the Proficiency Test Provider (260ppm)

$\text{Corrected Result}_{ppm}$ = calculated normalized result from Program's run (112.62, 759.52, 321.65, 191.01 respectively)

Table C.8—Conversion of Corrected Results to Uncorrected Results

	PT cylinder 1	PT cylinder 2	PT cylinder 3	PT cylinder 4
Corrected results (ppm)	112.62	759.52	321.65	191.01
Uncorrected results (g/210 L)	0.043	0.292	0.124	0.073

Step 5 – the Uncorrected results (g/210 L) will be reported to the PTP

Annex D
(informative)

Example of Validation Results[1]: Accuracy

Table D.1 provides an example of mock validation results related to bias and precision. This is for illustrative purposes only; results for a successful mock calibration are not provided.

Table D.1—Summary of Validation Results: Accuracy

METHOD 5Record Title:		Model X2021 Accuracy Validation Data			
Method Name: Location	Headquarters Laboratory		Sunset PD Breath Alcohol Calibration Method		
Date/initials:	08/15/16 DBS	08/15/16 DBS	08/15/16 DBS	08/15/16 DBS	08/15/16 DBS
Instrument SN	90320	90320	90320	90320	90320
CRM matrix	gas	gas	gas	aqueous	aqueous
CRM Manufacturer	ACME	ACME	ACME	DURHAM	DURHAM
CRM Lot#	AL141202	AL141208	AL141015	NC1030	NC1060
CRM Exp.	16-Dec	16-Dec	16-Oct	16-Oct	16-Nov
Target conc (g/210L)Ethanol Calibrator Range:	0.02		0.08	0.15	0.30 0.60 0.0150 – 0.350 g/210 L
Maximum concentration for low validation reference material:	0.045 g/210 L				
Minimum concentration for high validation reference material:	0.280 g/210 L				

[1] This is an example of mock Validation Results, for illustrative purposes only.

Replicate #1Validation concentrations for precision + bias:	0.019• 0.020 g/210 L • 0.150 g/210 L • 0.300 g/210 L	0.079	0.148	0.302	0.601

Low Nominal Quantity Value	0.020 g/210 L	Results				Within-Run Statistics			
Date	Initials	Location	Rep 1	Rep 2	Rep 3	Mean	Standard Deviation	Bias (g/210 L)	% CV
Replicate #2/10/2021	MG	HQ	0.019	0.07902	0.14902	0.304020	0.595001	0.000	2.9
Replicate #32/11/2021	SZ	Barrack 5	0.0200019	0.079018	0.150018	0.303018	0.599001	-0.002	3.1
Replicate #42/12/2021	0.020DR	0.080Barrack 2	0.14902	0.303021	0.602021	0.021	0.001	0.001	2.8
Replicate #5	0.020	0.080	0.150	0.303	0.605				
Replicate #62/16/2021	CF	Barrack 8	0.018	0.02	0.02	0.019	0.080001	-0.151001	6.0.304
Replicate #72/18/2021	0.020BS	0.081Barrack 3	0.150017	0.303018	0.598018	0.018	0.001	-0.002	3.3
Replicate #82/22/2021	MK	HQ	0.018	0.019	0.081021	0.149019	0.304002	-0.599001	7.9
Replicate #9	0.020	0.080	0.150	0.304	0.601				
Replicate #10	0.020	0.081	0.150	0.304	0.602				
Mean:	0.020	0.080	0.150	0.303	0.600				

~~Range (Low-High):~~	Between-Run Statistics			Grand Mean	0.019~~-0.020~~	~~0.079-0.081~~	~~0.148-0.151~~	~~0.302-0.304~~	~~0.595-0.602~~
				~~Calculated~~ Bias ~~%~~(g/210 L)	~~–2.000~~0.001	0.000	~~-0.267~~	~~1.133~~	~~-0.050~~
~~(± 5% or 0.005) acceptable bias range~~	~~0.015-0.025~~	~~0.075-0.085~~	~~0.1425-0.1575~~	~~0.285-0.315~~	~~0.570-0.630~~				
				Standard Deviation	0.001	~~0.001~~	~~0.001~~	~~0.001~~	~~0.003~~
~~Acceptable bias and precision~~	~~Yes~~			~~Yes~~% CV	~~Yes~~6.5	~~Yes~~		~~Yes~~	

Middle Nominal Quantity Value	0.150 g/210 L	Results				Within-Run Statistics			
Date	Initials	Location	Rep 1	Rep 2	Rep 3	Mean	Standard Deviation	Bias (%)	% CV
2/10/2021	MG	HQ	0.148	0.149	0.15	0.149	0.001	-0.7	0.7
2/11/2021	SZ	Barrack 5	0.145	0.147	0.148	0.147	0.002	-2.2	1.0
2/12/2021	DR	Barrack 2	0.15	0.149	0.151	0.150	0.001	0.0	0.7
2/16/2021	CF	Barrack 8	0.147	0.149	0.15	0.149	0.002	-0.9	1.0
2/18/2021	BS	Barrack 3	0.146	0.148	0.151	0.148	0.003	-1.1	1.7
2/22/2021	MK	HQ	0.152	0.151	0.153	0.152	0.001	1.3	0.7
					Grand Mean	0.15			
					Bias (%)	-0.59			
		Between-Run Statistics			Standard Deviation	0.002			
					% CV	1.4			

High Nominal Quantity Value	0.300 g/210 L	Results				Within-Run Statistics			
Date	Initials	Location	Rep 1	Rep 2	Rep 3	Mean	Standard Deviation	Bias (%)	% CV
2/10/2021	MG	HQ	0.305	0.309	0.307	0.307	0.002	2.3333	0.7
2/11/2021	SZ	Barrack 5	0.302	0.304	0.303	0.303	0.001	1.0000	0.3
2/12/2021	DR	Barrack 2	0.297	0.299	0.3	0.299	0.002	-0.4444	0.5
2/16/2021	CF	Barrack 8	0.301	0.302	0.301	0.301	0.001	0.4444	0.2
2/18/2021	BS	Barrack 3	0.298	0.299	0.298	0.298	0.001	-0.5556	0.2
2/22/2021	MK	HQ	0.3	0.302	0.304	0.302	0.002	0.6667	0.7
					Grand Mean	0.30			
					Bias (%)	0.57			
		Between-Run Statistics			Standard Deviation	0.003			
					% CV	1.1			

Annex E
(informative)

Example of ~~Additional~~ Validation ~~Parameters and~~ Results ~~–~~: Reference Material Part 1[m]

E.1 Freeze/Thaw Validation Plan

E.1.1 Validation Plan:

To evaluate the effect of freezing and thawing on ~~wet bath~~aqueous calibration ~~materials~~material, the Program will examine the effect on 0.080 g/210L reference ~~materials~~material. A total of twenty bottles of reference ~~materials~~material from the same lot will be used in this experiment. Five bottles (e.g., ~~bottle~~bottles 1~~-~~ through 5) that have never been frozen will be analyzed initially five times (replicates). The remaining bottles (e.g., ~~bottle~~bottles 6~~-~~ through 20) will then be placed in a freezer at –10˚C for a minimum of forty-eight hours. After they are allowed to thaw unassisted at room temperature for forty-eight hours, five bottles (e.g., ~~bottle~~bottles 6~~-~~ through 10) will be analyzed five times (replicates). The remaining bottles will undergo a second (e.g., ~~bottle~~bottles 11~~-~~ through 15) and third (e.g., ~~bottle~~bottles 16~~-~~ through 20) freeze/thaw cycle respectively, and be analyzed in the same fashion described previously.

This experiment will be performed on an "ABC-123" instrument with "XYZ" ~~Simulators~~simulators connected to the instrument's calibration port. All reference ~~materials~~material shall be of the same lot for adequate comparison.

E.1.2 Acceptance Criteria:

The reference ~~materials~~material are considered stable if the combined mean of the analysis of the frozen/thawed samples ~~is within 5% of~~meets the ~~combined mean of the initial analysis~~bias (0.005 g/210 L) and precision (± 10 % CV) acceptance criteria.

E.2 Freeze/Thaw Validation Results and Summary

The validation experiment took place from 02/01/2016 to 02/27/2016 using 0.080 g/210L reference material lot 3456-8. The raw data and all documentation generated from this experiment have been retained in the validation file for this method. The mean of each analysis, as well as the combined mean for each event are recorded in the ~~following table~~Table E.1.

Table E.1—Freeze/Thaw Experiment Data ~~Means~~

	Initial Analysis	After One Cycle	After Two Cycles	After Three Cycles
	Bottles 1-5	Bottles 6-10	Bottles 11-15	Bottles 16-20
Replicate Mean	0.0813	0.0809	0.0805	0.0798
Replicate Mean	0.0807	0.0805	0.0800	0.0795
Replicate Mean	0.0817	0.0815	0.0811	0.0804

[m] This is an example of additional mock Validation Parameters and subsequent results, for illustrative purposes only.

	Initial Analysis	After One Cycle	After Two Cycles	After Three Cycles
Replicate Mean	0.0809	0.0806	0.0802	0.0797
Replicate Mean	0.0811	0.0809	0.0806	0.0801
~~Combined~~Within-run Mean:	0.0811	0.~~0808~~0809	0.0805	0.0799
Within-run SD:	0.00038	0.00039	0.00042	0.00035
Bias (%)	0.2	-0.1	-0.6	-1.4
% ~~Deviation~~CV	0.47	0.~~37%~~48	0.~~74%~~52	~~1.48%~~0.44
~~<5% of combined mean?~~		~~Yes~~	~~Yes~~	~~Yes~~

The impact of freeze/thaw cycles was demonstrated to be at an acceptable level, with the greatest ~~deviation from the initial analysis~~change in bias being a loss of 1.~~48~~4%, which meets the requirement to be less than 5%. However, the lab noted ~~that the deviation consistently increased slightly~~an increasing negative bias with each freeze/thaw cycle; therefore, a decision was made to discard any reference material that goes through more than three freeze/thaw cycles.

Annex F
(informative)

Example of Additional Validation Parameters and Results: Reference Material – Part 2[n]

F.1 Minimum Allowable ~~psi~~Pressure of ~~Dry~~Compressed Gas Reference Standards

F.1.1 General

The purpose of this validation is to ensure that ~~dry~~compressed gas reference standards used for calibration of evidential Breath Alcohol instruments continue to provide acceptable results at minimum ~~psi (pounds per square inch)~~pressure (i.e., running low to empty). Anytown, USA calibrates 2 models of evidential instruments (Desktop A and Handheld B). The software used in Anytown, USA's evidential instruments will provide a "~~dry~~compressed gas tank empty" test result when the pressure reaches 50 psi (pounds per square inch) and will not allow a subject test or calibration to continue.

F.1.2 Validation Plan

Two instruments, installed with software version XXXXX will be used to assess Certified Reference Material (CRM) acceptability.

a) Test a minimum of three different ~~dry~~compressed gas CRM of different known values with a pressure close to 50psi; e.g., 60psi.

b) Record the initial pressure in psi for each CRM.

   1) Perform a test to determine if ~~it~~the result is within acceptable parameters (0.005 or +/-5%).

   2) Record the ~~psi~~pressure after this test.

c) Continue to perform tests of each CRM until:

   1) The instrument's minimum ~~psi~~pressure level of acceptance is reached (tank empty message appears), or

   2) An insufficient sample warning is given, or

   3) A result outside of the acceptable parameters is produced.

d) Perform a test on each CRM on different days and with different analysts, if possible.

e) Record the route of delivery for each test.

   1) Breath port.

[n] This is an example of additional mock Validation Parameters and subsequent results, for illustrative purposes only.

~~2) As an accuracy check with an internal pressure gauge~~

2) Reference material inlet.

Evaluate the results to determine the acceptable lower ~~psi~~pressure levels. Acceptability shall be concluded if CRM results are within the acceptable parameters at all concentrations and all pressure readings unless an instrument message alerts the user of "~~dry~~compressed gas tank empty".

F.1.3 Validation Results

Table F.1—Summary of Minimum Allowable ~~psi~~Pressure

Date/initials:	**2/11/16 ESZ**	**2/12/16 RPM**	**3/7/16 RPM**	**3/8/16 ESZ**	**3/9/16 ESZ**	**3/10/16 RPM**
Instrument Model	Desktop A	Handheld B	Desktop A	Handheld B	Desktop A	Handheld B
Instrument SN	1234	9876	1236	9874	1238	9872
Delivery Route	~~Internal~~Inlet	Breath port	~~Internal~~Inlet	Breath port	~~Internal~~Inlet	Breath port
Dry Gas Std. Lot/Tank #	OP416120/5	OP416121/3	OP335123/8	OP335126/7	OP435127/16	OP435128/12
Dry Gas Std. Exp.	06/10/2016	6/10/2016	12/19/2016	12/19/2016	12/19/2016	12/19/2016
~~Target~~Nominal quantity conc (g/210L)	**0.198**	**0.200**	**0.040**	**0.040**	**0.081**	**0.081**
Replicate #1	0.199	0.203	0.041	0.040	0.080	0.081
Replicate #1 psi	56	54	56	56	54	52
Replicate #2	0.199	0.204	0.041	0.040	0.080	0.081
Replicate #2 psi	51	54	54	55	54	52
Replicate #3	0.199	0.203	0.041	0.040	0.080	0.081
Replicate #3 psi	51	51	51	53	51	51
Replicate #4	0.199	0.198	0.041	0.040	0.080	0.081
Replicate #4 psi	51	46	51	46	51	46
Replicate #5	"Tank Empty"	*Sample Timeout	"Tank Empty"	*Sample Timeout	"Tank Empty"	*Sample Timeout
Replicate #5 psi	46	31	46	41	46	41
Acceptable Results (Y/N)	Yes	Yes	Yes	Yes	Yes	Yes

F.1.4 Validation Summary

A total of 3 Desktop A and 3 Handheld B instruments installed with Anytown, USA software were evaluated at low ~~psi~~pressure levels with ~~CRMs~~CRM. The results presented in ~~E~~F.1.~~2~~3 (Table ~~E~~F.1 – Summary of Minimum Allowable ~~psi~~pressure) indicate that all results at lower ~~psi~~pressure were either within the acceptable parameters or generated an instrument message. This demonstrates that no negative effects are to be expected while calibrating the instrument using ~~dry~~compressed gas cylinders at lower pressure. The instruments will either produce valid results or stop

calibration activities. The results objectively support that the Certified Reference Material used to calibrate is acceptable at low ~~psi~~pressure levels.

Annex G
(informative)

Example of a Validation Summary for Environmental Impact[o]

Scope and Purpose:

The performance of the calibration method (SOP 4.3) was assessed under similar environmental conditions that are typically encountered during calibration. The Town Everywhere Sheriff's Department calibrates evidential instruments in the laboratory and field (external facilities and roadside). The Town Everywhere experiences great fluctuation in temperatures and humidity throughout the year. Additionally, the instruments located in various facilities across Town Everywhere are subject to different barometric pressures due to differing altitudes. To assess the performance of the calibration method, ~~Breath Alcohol program~~Program personnel shall calibrate the instrument(s) under these expected conditions and evaluate the resultant data to ensure the method's acceptability.

Example experimental design (evaluation plan)

Temperature:

The lowest and highest laboratory temperatures over three months were recorded with a ~~NIST~~SI-traceable reference thermometer. An instrument was calibrated using SOP 4.3 during the temperature highs and lows. Post-calibration, the following parameters were assessed: bias, precision, and carryover. Results met the defined criteria for acceptance. Data are included in the main summary for the SOP 4.3 calibration method.

Annual temperature data from the National Weather Service- Everywhere Office was obtained to determine reasonable high and low temperature expectations for roadside conditions. The average high temperature was 110°F (43°C) and the average low temperature was –50°F (–45°C). An administrative decision was made to limit field calibration to the temperature range of 32°F to 100°F (0°C to 38°C). An instrument was calibrated three times each using SOP 4.3 at 32°F (0°C) and at 100°F (38°C). Post-calibration, the following parameters were assessed: bias, precision, and carryover. Results met the defined criteria for acceptance. Data are included in the main summary for the SOP 4.3 calibration method.

Barometric Pressure:

Altitude ranges from Town Everywhere were obtained. The altitude ranged from sea level (~10 meters) to 5,000 feet (~457 meters). An instrument was calibrated at the highest and lowest point in Town Everywhere. Post-calibration, the following parameters were assessed: bias, precision, and carryover. Results met the defined criteria for acceptance. Data are included in the main summary for the SOP 4.3 calibration method.

[o] This is an example of a portion of a mock Validation Summary for illustrative purposes only.

RFI:

The manufacturer provided independent testing to internationally accepted electromagnetic compatibility (EMC) standards. In addition to this independent testing, Everywhere Sheriff's Office personnel activated their emergency communication devices (radios) during and after the SOP 4.3 calibration method was being performed. Personnel were in close proximity to the instruments during these experiments. Results met the defined criteria for acceptance. A passing result is one which either flags an RFI error, *or* provides a valid result whose value is not altered by more than the acceptable bias from the expected result. Data are included in the main summary for the SOP 4.3 calibration method.

Annex H
(informative)

Bibliography

This is not meant to be an all-inclusive list as ~~the group recognizes~~ other publications on this subject may exist. At the time this standard was drafted, these were the publications available for reference. Additionally, any mention of a particular software tool or vendor as part of this bibliography is purely incidental, and any inclusion does not imply endorsement.

For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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