

Washington State Patrol

TOXICOLOGY LABORATORY DIVISION

Calibration – Reference Materials Technical Manual

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1 TECHNICAL SERVICE PROGRAM

This manual describes the Technical Services Program of the Washington State Patrol's (WSP) Toxicology Laboratory Division (TLD) as it relates to its breath alcohol calibration functions.

The Toxicology Laboratory (Toxicology Lab) and the Breath Test Program (BTP) are both responsible for the breath alcohol calibration functions of the Forensic Laboratory Services Bureau (FLSB). The Toxicology Lab prepares and certifies two types of simulator solutions: the Quality Assurance Procedure (QAP) solutions and the External Standard solution. These solutions are then used by the BTP, where the QAP solutions are used to set and confirm the calibration of the evidentiary breath test instruments, and the External Standard solution is used to verify the accuracy and proper working order of the instruments as part of a field evidential breath test.

The purpose of this manual is to specify in detail the many policies and procedures that shall be followed in order for the TLD to fulfill its breath alcohol calibration responsibilities, namely the production and certification of breath alcohol reference materials.

The official version of this manual is the electronic version as it appears on the FLSB Sharepoint site (FLSB Portal). This manual covers all work done by responsible personnel, to include but not limited to work done in the TLD laboratory, in addition to duties outside the laboratory, whether in court, training venues, or anywhere else the duties of responsible personnel might be employed.

1.1 POLICY

The TLD will document its policies and procedures to the extent necessary to assure the quality of the calibration results. Compliance with pre-established and carefully designed policies and procedures is important to ensure the work product and services are accurate and fit-for-purpose. The policies and procedures outlined in this manual will be communicated to, available to, understood by, and implemented by the responsible personnel.

All calibration and related services performed by the TLD shall meet generally recognized standards of the forensic community and its accrediting organizations. Specifically, the TLD shall perform all calibration activities in accordance with the specified program policies and the ISO 17025:2005 accreditation standards.

All employees are required to familiarize themselves with this manual and implement the policies and procedures specified herein. In doing so, the TLD will maintain the highest level of expertise and analytical confidence for the criminal justice system and comply with the ISO 17025:2005 accreditation standards and ASCLD/LAB-*International* supplemental standards.

Any adjustments or deviations from the policies and procedures detailed in this manual must be approved by the State Toxicologist, the Quality Assurance (QA) Manager, or a Supervisor, and appropriately documented in the Batch Record.

1.2 DEFINITIONS

1.2.1 ACCURACY

The proximity of a measured value to a reference value.

1.2.2 BATCH FILE

A file containing documentation produced as a result of certifying either an external standard solution or QAP solutions. Records include the Simulator Solution Data Entry Review form, the QAP or External Standard Solution Test Report, the Solution Certificate Review, analyst affidavits/certifications, sequence tables and corresponding chromatograms, and the Solution Preparation Worksheet.

1.2.3 BATCH RECORD

All documentation related to the preparation and/or certification of either an external standard solution or QAP solutions. In addition to those records contained within the batch file, records may include simulator solution preparation log, alcohol preparation log, alcohol control log, instrument maintenance records, etc.

1.2.4 BIAS

The difference between a measurement result and the true reference value of the property being measured. The bias quantifies the accuracy of the measurement.

1.2.5 BREATH TEST TECHNICIANS / TECHNICIANS

Currently qualified Operators who are trained in the following areas of responsibility: instrument calibration, certification, repair, maintenance, documentation, training of operators and expert court testimony. Technicians are also qualified Instructors, and Solution Changers.

1.2.6 CALIBRATION

The process by which known traceable standards having reference values are introduced into an instrument. The instrument is then adjusted or programmed (either by software, hardware, electronics, etc.) to report a measurement based on the known reference value(s).

1.2.7 COEFFICIENT OF VARIATION (C.V.)

The relative standard deviation expressed as a percentage of the mean.

1.2.8 COMBINED UNCERTAINTY

The estimate of measurement uncertainty that includes the contribution from all components significantly influencing a measurement result

1.2.9 EXTERNAL STANDARD SOLUTION

The solution used within the simulator to provide a known alcohol vapor concentration to verify the accuracy and proper working order of the instrument as part of a field evidentiary breath test.

1.2.10 FORENSIC SCIENTISTS / ANALYSTS

Personnel trained and assigned to the Toxicology Lab for the purpose of solution preparation and certification.

1.2.11 NATIONAL INSTITUTE FOR STANDARDS AND TECHNOLOGY (NIST)

A federal agency located within the Department of Commerce with final authority for metrology in the United States.

1.2.12 PRECISION

The ability of a technique to perform a measurement in a reproducible manner. Precision is quantified by the standard deviation.

1.2.13 QUALITY ASSURANCE PROCEDURE (QAP)

A testing procedure for evidentiary breath test instruments in which known traceable reference materials are used to set and confirm the calibration and establish quantitative estimates for bias and precision. Several other performance measures are also evaluated in order to ensure the proper working order and evidential suitability of the instrument.

1.2.14 QUALITY ASSURANCE PROCEDURE (QAP) SOLUTION

The solution used within the simulator to provide a known alcohol vapor concentration to set and confirm the calibration of the evidentiary breath test instrument.

1.2.15 ROUNDING

When rounding is performed for computational purposes, normal rules of rounding are followed unless otherwise specified.

1.2.16 SIMULATOR

A device, when filled with a certified simulator solution maintained at a known temperature, that provides a vapor sample of a known ethanol concentration.

1.2.17 STANDARD UNCERTAINTY

The uncertainty of a measurement result expressed as a standard deviation.

1.2.18 TEST REPORT

The final result sheet produced at the end of either a QAP solution or external standard solution testing process. It includes ethanol concentrations from individual solution aliquots, ethanol control results, statistical data, signatures of the preparer and other certifying analysts, and dates of preparation testing and issuance.

1.2.19 TOXICOLOGY LABORATORY DIVISION (TLD) MANAGEMENT

Includes all scientific personnel in the chain of command at the level of Supervisor and above.

1.2.20 TRACEABILITY

The property of a measurement result whereby it can be related to standard references, usually national or international, through an unbroken chain of comparisons all having stated uncertainties.

1.2.21 UNCERTAINTY

A parameter, associated with a measurement result that characterizes the dispersion of the values that could reasonably be attributed to the true value being measured.

2 PREPARATION OF QUALITY ASSURANCE PROCEDURE (QAP) SOLUTIONS

2.1 POLICY

The Quality Assurance Procedure (QAP) solutions are a mixture of water and ethanol formulated to provide a standard ethanol vapor concentration when used in a breath alcohol simulator heated to 34.0 ± 0.2 °C. The QAP solutions are used to set and confirm the calibration of, and verify the accuracy and precision of, evidentiary breath test instruments.

The QAP program requires target vapor concentrations of 0.04, 0.08, 0.10 and 0.15 g/210L vapor. The reference value concentration of a given QAP solution is determined from replicate measurements by gas chromatography.

2.2 EQUIPMENT

- Volumetric glassware/flasks
- 2, 4, 6 L Erlenmeyer flask
- Mechanical mixer and stir rod
- 18 L containers
- Storage bottles/containers
- Tamper evident tape, or equivalent

2.3 REAGENTS

- 200 proof absolute ethanol (USP Grade)
- Laboratory grade deionized water

2.4 PROCEDURE

1. The preparer will assign a batch number to the QAP solution. The first two digits of the batch number represent the year in which the solution was made, followed by a sequential three-digit number, beginning with 001. Therefore, the first batch of 2008 would be 08001.
2. Prepare a Batch File marked with the batch number to store all relevant results and documents.
3. Using the Simulator Solution Preparation Log, record the batch number of the solution, the date of solution preparation, the preparer's name, the lot number of the absolute ethanol reagent, and the date this reagent was opened.
4. Use the values in Table 1 to prepare each QAP solution.

Table 1:	Target Vapor Concentration	Ethanol/Water Dilution Factor
	0.04	11.2 mL/18 L
	0.08	22.4 mL/18 L
	0.10	28.1 mL/18 L
	0.15	42.0 mL/18 L

5. Using an Erlenmeyer flask, fill the flask to approximately 80% of its nominal volume with deionized water.
6. Using volumetric glassware, add appropriate volume of absolute ethanol to the Erlenmeyer flask, as indicated in Table 1.
7. Fill the Erlenmeyer flask to the nominal mark with deionized water. Add the ethanol/water mixture to the 18 L vessel.
8. Fill the same Erlenmeyer flask to the nominal mark with deionized water and add this to the 18 L vessel. Repeat this step until a total of 18 L has been transferred.
9. Tighten the cap of the 18 L vessel. Mix the solution by applying mechanical mixing for a minimum of 30 minutes.
10. Once mixing is complete, purge the spigot then remove an aliquot of the solution for certification (*see 4 Certification of Simulator Solutions*).
11. Documentation of the preparation of the QAP solutions should be recorded on the Combined Simulator Solution Preparation Worksheet. This worksheet will be placed in the batch file.

2.5 PACKAGING AND DISTRIBUTION

1. The QAP solutions are provided in containers of convenient size.
2. Prior to filling, each container is labeled with "QAP", the batch number, the appropriate target vapor concentration, the preparer's initials, and the preparation and expiration date.
3. The containers are sealed with tamper evident tape, or equivalent.
4. Once the QAP solutions are certified and approved for use, they may be provided to breath test technicians for use with the breath test instruments.
5. Solutions are boxed and sent by Consolidated Mail Services, or equivalent.
6. Insert the Solution Request & Packing Slip. Retain a copy of the packing slip in the batch record.
7. The storage of QAP solutions in the Toxicology Lab will be in a secure location to protect against damage, deterioration or loss and to maintain the solutions' integrity.

Bottles will be stored in moderate temperatures in secured, limited-access locations similar to those under which they were produced.

2.6 RECEIPT AND STORAGE OF QAP SOLUTIONS

1. On receipt of the QAP Solutions, the Technician will sign and date the packing slip, indicating:
 - a. Verification of order – adequate amount, correct concentrations, etc
 - b. Inspection of bottles – no damage, leaking, broken seals, etc
 - c. Appropriate Test Report(s) included
 - d. Record of receipt
2. If any discrepancies are noted, the Technician should contact the Toxicology Lab. Discrepancies may include insufficient quantity of QAP solutions, incorrect concentration, damaged and/or leaking bottles, and broken seals. Based on the specific discrepancy, the Toxicology Lab will endeavor to resolve the issue to the satisfaction of the Technician. Any discrepancies and subsequent resolution will be documented in the batch record.
3. On receipt of the QAP solutions, the Technician should store them in a secure cabinet/closet separate from any volatile chemicals. Extreme heat should be avoided.
4. The QAP solutions are valid and approved for use for a period of one year from the date of preparation. QAP solutions that have expired shall be discarded. If expired solutions are retained for training purposes then each container must bear identification that reads “Training Purposes Only – Not for use in Calibration” and be stored separate from non-expired QAP and external standard solutions.
5. Expired solutions may be discarded down a drain with additional water, and the solution containers discarded in the trash or recycled.
6. When a QAP solution is transferred to a simulator, the simulator is to be labeled with the identity of the QAP solution, the QAP solution batch number and the in service date.

3 PREPARATION OF THE EXTERNAL STANDARD SOLUTION

3.1 POLICY

The External Standard solution is a mixture of water and ethanol formulated to provide a standard ethanol vapor concentration when used in a breath alcohol simulator heated to 34.0 ± 0.2 °C, of between 0.072 and 0.088 grams of ethanol per 210 liters (g/210L) of air, inclusive. To allow for depletion of ethanol from the solution during its use, the target starting ethanol vapor concentration is 0.082 g/210L.

Based on a water/air partition coefficient at 34 °C of 2585.9 (Jones, 1983), the external standard solution concentration required to produce a 0.082 g/210L of vapor equivalent is 0.101 g/100ml. The reference value concentration of a given external standard solution is determined from replicate measurements by gas chromatography.

3.2 EQUIPMENT

- Volumetric glassware/flasks
- Mechanical mixer and stir rod
- 52 L container
- Storage bottles/containers
- Tamper evident tape, or equivalent

3.3 REAGENTS

200 proof absolute ethanol (USP Grade)
Laboratory grade deionized water

3.4 PROCEDURE

1. The preparer will assign a batch number to the external standard solution. The first two digits of the batch number represent the year in which the solution was made, followed by a sequential three-digit number, beginning with 001. Therefore, the first batch of 2008 would be 08001.
2. Prepare a Batch File marked with the batch number to store all relevant results and documents.
3. Using the Simulator Solution Preparation Log, record the batch number of the solution, the date of solution preparation, the preparer's name, the lot number of the absolute ethanol reagent, and the date this reagent was opened.
4. Fill the 52 L vessel to approximately 80% of the 52 L mark with deionized water.
5. In a volumetric flask, fill to approximately 50% with deionized water. Using volumetric glassware, add 66.5 mL of absolute ethanol. Stopper the flask and mix well and add the contents of the flask to the 52 L vessel. Rinse the flask with deionized water and add this to the 52 L vessel.

6. Fill the vessel to 52 L with deionized water and tighten the cap. Mix the solution by applying mechanical mixing for a minimum of two hours.
7. Once mixing is complete, purge the spigot then remove an aliquot of the solution for certification (*refer to 4.0 Certification of Simulator Solutions*).
8. Documentation of the preparation of the external standard solution should be recorded on the Combined Simulator Solution Preparation Worksheet. This worksheet will be placed in the batch file.

3.5 PACKAGING AND DISTRIBUTION

1. The external standard solution is provided in containers of convenient size.
2. Prior to filling, each container is labeled with “External Standard”, “ESS” or “Ext. Std.”, the batch number, the appropriate target vapor concentration, the preparer’s initials, and the preparation and expiration date.
3. The containers are sealed with tamper evident tape, or equivalent.
4. Once the external standard solution is certified and approved for use, it may be provided to breath test technicians for use with the breath test instruments.
5. Solutions are boxed and sent by Consolidated Mail Services, or equivalent.
6. Insert the Solution Request & Packing Slip. Retain a copy of the packing slip in the batch record.
7. The storage of external standard solutions in the Toxicology Lab will be in a secure location to protect against damage, deterioration or loss and to maintain the solutions’ integrity. Bottles will be stored in moderate temperatures in secured, limited-access locations similar to those under which they were produced.

3.6 RECEIPT AND STORAGE OF EXTERNAL STANDARD SOLUTIONS

1. On receipt of the external standard solutions, the Technician will sign and date the packing slip, indicating:
 - a. Verification of order – correct amount, correct concentrations, etc
 - b. Inspection of bottles – no damage, leaking, broken seals, etc
 - c. Appropriate Test Report included
 - d. Record of receipt
2. If any discrepancies are noted, the Technician should contact the Toxicology Lab. Discrepancies may include insufficient quantity of external standard solutions, incorrect concentration, damaged and/or leaking bottles, and broken seals. Based on the specific discrepancy, the Toxicology Lab will endeavor to resolve the issue to the satisfaction of the Technician. Any discrepancies and subsequent resolution will be documented in the Batch Record.

3. On receipt of the external standard solutions, the technician should store them in a secure cabinet/closet separate from any volatile chemicals. Extreme heat should be avoided.
4. The external standard solutions are valid and approved for use for a period of one year from the date of preparation. External standard solutions that have expired shall be discarded. If expired solutions are retained for training purposes then each container must bear identification that reads "Training Purposes Only – Not for use in Calibration" and be stored separate from non-expired external standard and QAP solutions.
5. Expired solutions may be discarded down a drain with additional water, and the solution containers discarded in the trash or recycled.

3.7 REFERENCE(S)

A.W. Jones. Determination of Liquid/Air Partition Coefficients for Dilute Solutions of Ethanol in Water, Whole Blood and Plasma. *Journal of Analytical Toxicology*, 7, 1983 pp 193-197.

4 CERTIFICATION OF SIMULATOR SOLUTIONS

4.1 POLICY

Each external standard and QAP solution must be certified by forensic scientists prior to its distribution to breath test technicians. The forensic scientists must have a valid Blood Alcohol Analyst Permit issued by the State Toxicologist.

A minimum of three (3) analysts shall test each solution before the average solution concentration can be calculated. Typically, three (3) analysts certify each set of QAP solutions, and seven to eight (7-8) analysts certify the external standard solution. Each analyst who has results included in the final computation of the average solution concentration has certified the batch.

Batches that do not certify as specified below are not approved for use and a Test Report is not generated. However, a batch record and batch file are still produced, including documentation of why the batch did not certify.

Any adjustments or deviations from the procedures below must be approved by TLD Management, and appropriately documented in the batch file.

4.2 EQUIPMENT

- Balance: Mettler Toledo PL602-S, or equivalent
- Volumetric glassware
- Class A Pipettes
- Storage bottles/containers
- Microlab 500 Autopipette, Hamilton Automatic Diluter, or equivalent
- Headspace autosampler vials, 10 mL
- Headspace autosampler crimp tops
- Cap crimper
- Cap de-crimper
- Agilent (Hewlett Packard) 7694/G1888 Headspace Autosampler or equivalent
- Agilent (Hewlett Packard) 6890 gas chromatograph; equipped with a J&W DBALC1 capillary column (30 m x 0.53 mm ID x 3 μ m) and/or with a J&W DBALC2 capillary column (30 m x 0.53 mm ID x 2 μ m), or equivalent
- Computer System equipped with Agilent (Hewlett Packard) ChemStation Software

4.3 REAGENTS

- 1-Propanol
- Sodium chloride
- 200 proof absolute ethanol (USP Grade)
- Laboratory grade deionized water (d.H₂O)
- Compressed air, helium and hydrogen

4.3.1 INTERNAL STANDARD

The Internal Standard (ISTD) is prepared and verified according to the Laboratory's Procedure for the Verification of n-Propanol Internal Standard.

Verification of the ISTD is documented on the Alcohol Standard Preparation Log and the Combined Ethanol Verification Worksheet.

Verification is required prior to use.

4.3.2 ETHANOL CALIBRATORS

Three ethanol calibrators (CAL) are used, at concentrations of: 0.079, 0.158, and 0.316 g/100 mL

Ethanol calibrators are prepared and verified according to the Laboratory's Procedure for Verification of Ethanol Calibrators.

Verification of the Ethanol Calibrators is documented on the Alcohol Standard Preparation Log and the Combined Ethanol Verification Worksheet.

Verification is required prior to use.

4.4 CONTROLS

Commercially prepared ethanol controls (CTRL) are purchased for use with each assay. The source and lot number of each control is documented in the Alcohol Control Log. The ethanol controls are verified according to the instructions on the Combined Ethanol Verification Worksheet. Verification is required prior to use. Controls are stored per manufacturer specifications.

Three ethanol controls are used, at concentrations of:

CTRL1 0.04 g/100 mL
CTRL2 0.10 g/100 mL
CTRL3 0.20 g/100 mL

Ethanol controls are considered approved for use when quantifying within the following, inclusive ranges.

CTRL1 0.038 – 0.042 g/100 mL
CTRL2 0.095 – 0.105 g/100 mL
CTRL3 0.190 – 0.210 g/100 mL

Controls other than the aforementioned may be approved for use by TLD Management, with appropriate documentation.

4.5 PROCEDURE FOR THE ANALYSIS OF SIMULATOR SOLUTIONS

The analyst who prepared the solution(s), and each subsequent analyst, will analyze five samplings of the aliquot taken from the original mixture (either 18 or 52 L).

External Standard Solution batches should be set up using the following sequence:

1. Blank (d.H2O, no Internal Standard added)	9. Negative Control
2. CAL 1 (0.079 g/100 mL)	10. Solution aliquot #1
3. CAL 2 (0.158 g/100 mL)	11. Solution aliquot #2
4. CAL 3 (0.316 g/100 mL)	12. Solution aliquot #3
5. Negative Control (d.H2O plus Internal Standard)	13. Solution aliquot #4
6. Control 1 (0.04 g/100 mL)	14. Solution aliquot #5
7. Control 2 (0.10 g/100 mL)	15. Control 0.10 g/100 mL
8. Control 3 (0.20 g/100 mL)	16. Negative Control

QAP Solution batches should be set up using the following sequence:

1. Blank (d.H2O, no Internal Standard added)	20. QAP 0.08 aliquot #4
2. CAL 1 (0.079 g/100 mL)	21. QAP 0.08 aliquot #5
3. CAL 2 (0.158 g/100 mL)	22. Control 0.10 g/100 mL
4. CAL 3 (0.316 g/100 mL)	23. Negative Control
5. Negative Control (d.H2O plus Internal Standard)	24. QAP 0.10 aliquot #1
6. Control 1 (0.04 g/100 mL)	25. QAP 0.10 aliquot #2
7. Control 2 (0.10 g/100 mL)	26. QAP 0.10 aliquot #3
8. Control 3 (0.20 g/100 mL)	27. QAP 0.10 aliquot #4
9. Negative Control	28. QAP 0.10 aliquot #5
10. QAP 0.04 aliquot #1	29. Control 0.10 g/100 mL
11. QAP 0.04 aliquot #2	30. Negative Control
12. QAP 0.04 aliquot #3	31. QAP 0.15 aliquot #1
13. QAP 0.04 aliquot #4	32. QAP 0.15 aliquot #2
14. QAP 0.04 aliquot #5	33. QAP 0.15 aliquot #3
15. Control 0.10 g/100 mL	34. QAP 0.15 aliquot #4
16. Negative Control	35. QAP 0.15 aliquot #5
17. QAP 0.08 aliquot #1	36. Control 0.10 g/100 mL
18. QAP 0.08 aliquot #2	37. Negative Control
19. QAP 0.08 aliquot #3	

1. Using the Auto-pipetter, extract 200 µL of the calibrators, controls or simulator solution and 2 mL of the internal standard solution.
2. Elute the aliquot/extract into a clean, labeled 10 mL headspace vial.
3. Seal the vial tightly.
4. Between each aliquot/extract, rinse and wash the pipette tip appropriately (e.g. rinse pipette tip with diluted bleach and/or d.H₂O. Repeat if necessary). It is not necessary to rinse and wash the pipette tip in-between repeated aliquots from a single simulator solution.

5. Load and edit a sequence on the headspace gas chromatograph. Enter the blank, calibrators, controls and simulator solutions into the sequence table, and identify them appropriately under Sample Type.
6. Place each headspace vial in the appropriate position on the headspace autosampler and verify this placement against the sequence log.
7. Run sequence under method SIMALC. [Note: The method may contain a numeric suffix to differentiate between instruments; for example SIMALC1 for headspace instrument 1. All certification testing for a given simulator solution will take place on a single instrument.]
8. Upon completion of testing, analysts will initial their chromatograms and sequence table.

If two or more separate external standard solution batches are prepared close together, each batch may be certified using the same calibration and controls. For the analysis of multiple external standard solution batches and QAP solution batches, each set of 5 aliquots should be separated by a 0.10 g/100 mL control and a negative control. It is the 0.10 g/100 mL control run at the end of each set of 5 aliquots that is entered into the database.

4.6 ACCEPTANCE PARAMETERS

If the analysis of the batch meets the criteria listed below, the results for the simulator solution(s) are accepted.

- Ensure that the blank is devoid of any significant peaks
- Ensure that the negative control is devoid of any significant peaks other than the internal standard. Should the negative control read above 0.005 g/100 mL for ethanol, the analyst re-aliquots and reanalyzes their sequence
- Verify that each calibrator and control quantifies to within $\pm 10\%$ of the target values. Should one of the calibrators or controls read outside $\pm 10\%$ for ethanol, the analyst re-aliquots and reanalyzes their sequence
- Each individual external standard solution result must be within the range 0.096-0.106 g/100 mL, inclusive.
- Each individual QAP solution result must be within the ranges specified in Table 2

Table 2:	Target Vapor Concentration	Equivalent Solution Concentration	Acceptable Range (inclusive)
	0.04	0.049	0.047 - 0.051
	0.08	0.098	0.093 - 0.103
	0.10	0.123	0.117 - 0.129
	0.15	0.185	0.176 - 0.194

- Should any individual value be outside of the specified range, the analyst re-aliquots and reanalyzes their sequence. The original testing results will be retained but not used in calculations. If, in the course of testing a batch, two or more individual values are outside of the specified range, either during original analysis or re-analysis, then the batch will not be certified.

4.7 CERTIFICATION, DOCUMENTATION AND REVIEW

1. Analysts will place their chromatograms and sequence tables in the batch file. When all batch testing has been completed, a supervisor will generate the appropriate Solution Test Report which includes the average solution concentration (arithmetic mean) rounded to four decimal places, the standard deviation rounded to five decimal places, and the percent coefficient of variation rounded to two decimal places.
2. The solution meets the standards required by the State Toxicologist if:
 - i. For the external standard solution, the average solution concentration (final arithmetic mean) is within the range 0.096 – 0.106 g/100mL, inclusive
 - ii. For the QAP solutions, the average solution concentration (final arithmetic mean) is within the ranges specified in Table 3

Table 3:

Target Vapor Concentration	Equivalent Solution Concentration	Acceptable Range (inclusive)
0.04	0.049	0.047 - 0.051
0.08	0.098	0.093 - 0.103
0.10	0.123	0.117 - 0.129
0.15	0.185	0.176 - 0.194

- iii. The CV is 5% or less
3. The equivalent vapor concentration is calculated by dividing the final average solution concentration by 1.23 and rounding to four decimal places.
 4. For an external standard solution, the expanded uncertainty is calculated based on the Division’s policy for estimating the combined uncertainty of external standard solutions.
 5. The batch file will be forwarded to a Toxicology Lab supervisor or designee for technical review. At this stage, the batch file should contain the printed Test Report, the chromatograms and sequence tables, and the Solution Preparation Worksheet.

The supervisor or designee will verify all preparation and testing dates are correctly documented, the ethanol control expiration dates have not been exceeded, individual chromatograms are initialed, all pages of the record are labeled with the batch number, the correct ethanol concentrations were entered into the Test Report, and the calibrators and controls were within the acceptable range. The appropriate QAP

or ESS Test Report Calculation Record will be produced to document performance of calculations and technical review of the entire batch file.

Any discrepancies identified in the review process will be brought to the attention of the analyst performing the work who will resolve them as soon as possible. Discrepancies not resolved at this level will be brought to the attention of the QA Manager and/or other TLD Management as appropriate.

6. Upon completion of the technical review, the batch file is returned to the analysts.

Each analyst should again verify that the preparation/testing dates and the data from their chromatograms correctly appear on the printed Test Report before signing on the corresponding signature line. Their signature will also reflect that the results are the results of tests that they personally performed.

Each analyst who certified the batch will also sign an affidavit as described in CrRLJ 6.13(c)(1), certification of simulator solution. Affidavits are placed in the batch file.

7. A technical and administrative review of the batch file will be performed by the Quality Assurance Manager or designee. The Quality Assurance Manager or designee will verify all preparation and testing dates are correct, chromatogram data is entered correctly, all chromatograms are included, accuracy and precision requirements are met, affidavits are signed and properly dated, etc. This review will be documented on the Simulator Solution Data Entry Review form, which will be added to the batch file.
8. Final solution calculations will be independently verified by the Quality Assurance Manager or designee and this verification documented on the Simulator Solution Data Entry Review form. Solution uncertainty calculations will also be verified at this time and the verification documented on the QAP or ESS Test Report Calculation Record.
9. A Toxicology Lab supervisor or designee will then perform a final administrative review and will sign and date the bottom of the Test Report indicating that the batch file is complete and the above procedures have been reviewed. The final review date will be the issue date of the Test Report and the batch. Simulator Solutions must not be distributed for use prior to this issue date.
10. The final batch file should contain:
 - i. The original QAP or External Standard Test Report, signed by each analyst
 - ii. The Solution Certificate Review form
 - iii. Analyst's affidavits
 - iv. All relevant sequence tables and chromatograms
 - v. The Solution Preparation Worksheet
 - vi. The QAP or ESS Test Report Calculation Record

vii. The Simulator Solution Data Entry Review Form

11. The final batch file shall be uploaded to the WSP Breath Test Program's discovery material website (i.e. WebDMS, or however named). The batch file shall be scanned as a pdf file and named with the batch number (e.g. 12001.pdf). The pdf file is then uploaded to the Solution Batch Certifications section of the WebDMS site, using the WebDMS Administrative Console by a TLD Supervisor or designee.

5 TRACEABILITY

5.1 POLICY

Traceability is established for measurement results, not for laboratories, methods or personnel. Traceability will be established for the individual measurement results and the mean calculations resulting from all results generated within the TLD. Traceability should establish an unbroken chain of comparisons for these measurement results back to national or international measurement standards such as NIST. Traceability will allow for comparability between different analytical instruments and methods.

5.2 PROCEDURE

1. All measurement results, mean calculations, batch numbers, and reference values will be recorded on the appropriate forms.
2. The Simulator Solution Test Report issued by the Toxicology Lab will record the simulator solution batch number along with all measurement results obtained by the analysts in the Toxicology Lab. The Report will also contain the results of control measurements along with the control lot number and reference value. One control measurement shall be performed along with the set of five aliquots of the simulator solution. All control measurements performed shall be within $\pm 10\%$ of the control reference value which will ensure the accuracy of the gas chromatograph instrument and the resulting reference value assigned to the simulator solutions.
3. The Toxicology Lab shall obtain and maintain a Certificate of Analysis (COA) from the reference material producer of the controls they purchase to be used during the testing of simulator solutions. The COA shall specify the lot number and reference value assigned to the purchased control solutions. The COA should also specify that the measurements performed by the manufacturer of the controls have been performed by methods and equipment that also measured Standard Reference Materials obtained from NIST.
4. The following two documents shall document and ensure traceability:
 - a. The COA from the commercial manufacturer of the controls
 - b. The Simulator Solution Test Report
5. The traceability links will be from:
 - a. The measurement results and mean reported on the Simulator Solution Test Report to:
 - b. The control measurement results along with lot number and reference value for the controls reported on the Simulator Solution Test Report to:
 - c. NIST as documented on the Certificate of Analysis from the control manufacturer, where applicable.

6 PROFICIENCY TEST PROGRAM

6.1 POLICY

Each forensic scientist within the TLD will complete at least one external proficiency test per year. All forensic scientists will be trained on the importance and procedures for proficiency testing as outlined in this policy. The training will include the procedures to be followed as well as forms to be completed. The purpose of proficiency testing will be to ensure the overall program's fitness-for-purpose.

The objectives of the proficiency testing program are to:

- Demonstrate the current competence of the analysts
- Demonstrate the current competence of the program
- Ensure that quality work is being performed and maintained
- Identify areas where additional training or resources would be beneficial
- Verify the validity of technical procedures

Proficiency test samples (e.g. simulator solutions, blood) will be handled by analysts in a similar manner to those samples routinely received for calibration and/or testing purposes.

The QA Manager will oversee the Proficiency Testing Program for the TLD, including assigning proficiencies to all personnel, submitting results, maintaining records, and notifying individual personnel and the relevant Supervisor and/or the State Toxicologist of proficiency test results.

6.2 DEFINITIONS

6.2.1 ASCLD/LAB

The American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB). An organization that offers accreditation under the ASCLD/LAB-*International* program, which is based on the ISO 17025 standards and the ASCLD/LAB-*International* Supplemental Requirements.

6.2.2 APPROVED PROFICIENCY TEST PROVIDER

An individual, organization or company which has applied for and obtained approval from ASCLD/LAB to prepare and provide proficiency tests to participating forensic laboratories, in the forensic disciplines for which the provider has been approved.

6.2.3 PROFICIENCY TEST

A proficiency test is an internal or external test that is provided to evaluate the capability of analysts, technical support personnel and the overall quality performance of a laboratory.

6.2.4 PROFICIENCY TEST REVIEW COMMITTEE (PRC)

A committee of individuals appointed by the Board of ASCLD/LAB, because of their experience and expertise, to give guidance to ASCLD/LAB in the proficiency testing program for specific forensic disciplines.

6.2.5 PROFICIENCY TEST MATERIAL/SAMPLE

For the Toxicology Lab, proficiency test material may include blood or simulator solutions obtained either from an Approved Proficiency Test Provider or from within the Toxicology Lab. The State Toxicologist and/or the QA Manager may approve other types and sources of proficiency test samples.

6.3 PROCEDURE

1. Toxicology Lab

a. Proficiency Testing Process – External proficiency tests

Proficiency test samples will be provided to the analysts. A written protocol and data entry form from the Approved Proficiency Test Provider (or equivalent) will also be provided. The analyst will be directed to follow the protocol and documentation steps as outlined. The testing will be completed within the directed time period and documentation provided back to the QA Manager. Normal procedures for the technical and administrative review of results will apply. The QA Manager or designee will forward the final documentation to the Proficiency Test Provider (or equivalent).

b. Proficiency Testing Process – Internal proficiency tests

Internal proficiency tests may be prepared by the Toxicology Lab, independently to the analyst(s) being proficiency tested. Protocols for the preparation and certification of Simulator Solutions will be similar to those outlined in Chapter 2. The final arithmetic mean will be the reference value for that proficiency test solution. Protocols for the preparation of blood proficiency samples will be documented and retained by the QA Manager. Records of the test results performed in the Toxicology Lab will be maintained which identify these samples for proficiency test purposes.

One bottle, blood vial/tube or aliquot of the proficiency test sample will be provided to the analyst(s). A written protocol and data collection form will also be provided. Five aliquots of a simulator solution proficiency will be tested, while blood proficiency samples will be tested in duplicate. The results will be recorded on the collection form provided. The forms will be signed and dated by the responsible analyst and sent along with the corresponding documents to a technical reviewer. Normal procedures for the technical and administrative review of results will apply prior to sending the results to the QA Manager.

c. Results

For external proficiency tests, individual analyst results are typically compared to the summary results of all participants provided by the Provider.

For internal proficiency tests, the arithmetic mean of the proficiency samples shall be compared to the arithmetic mean determined independently by the Toxicology Lab. The mean of each analyst's results should typically be within ± 5 % of the pre-determined mean.

Additional statistical criteria may be applied to proficiency tests and will be documented and communicated to the analysts prior to testing.

2. Discrepancies and Non-Conformities

Procedures for proficiency test discrepancies and non-conformities are outlined in the TLD Calibration Quality Manual.

7 ESTIMATION OF UNCERTAINTY OF MEASUREMENT

7.1 POLICY

Uncertainty of measurement will be estimated for the values assigned to breath alcohol reference materials. The TLD has attempted to identify all the components of uncertainty contributing to this activity and has made reasonable estimates of each component for inclusion in its uncertainty budget. The estimation of uncertainty does not replace any existing policies established for the maintenance of quality control nor does it supersede any established legal, statutory or regulatory guidance on breath alcohol testing.

Uncertainty is not synonymous with error, inaccuracy or bias. Restrictions on measurement error have been integrated into the procedures for reference material certification and instrument calibration. Refer to Chapter 4 of the Calibration Technical Manual for a description of these restrictions.

This policy applies only to the functions of the TLD's breath alcohol calibration program as defined in its scope of accreditation.

7.2 UNCERTAINTY BUDGET

An uncertainty budget describes those components that have been identified as contributing to the overall measurement uncertainty for a given calibration activity. These components include contributions from reference standards, inexact values of reference materials, equipment used, approximations in the measurement procedure, inexact values of constants and variations in repeated observations (repeatability). Multiple sources may contribute to a single uncertainty component. When a component is estimated from a source external to the TLD, it is first converted to its standard uncertainty based on the reported coverage factor.

Figure 1 is a cause and effect diagram showing the uncertainty sources incorporated into the budget for the breath alcohol reference materials. It applies to both the external standard solution and the quality assurance procedure solutions. There are four major components that contribute to the overall uncertainty and they are broadly categorized as: analytical, repeatability, reference materials and external constants.

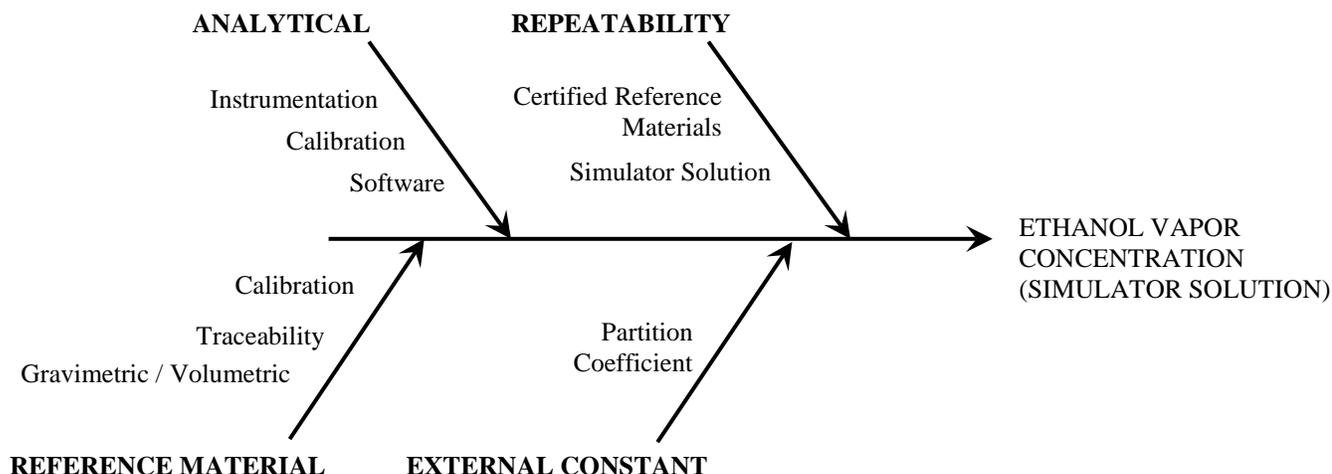


Figure 1: Cause and effect diagram for the breath alcohol calibration reference materials

7.3 MEASUREMENT UNCERTAINTY OF BREATH ALCOHOL CALIBRATION REFERENCE MATERIALS

7.3.1 UNCERTAINTY OF ETHANOL REFERENCE MATERIAL VALUE

Multiple ethanol reference materials are measured alongside a simulator solution during certification. The reference materials contain ethanol at a reference concentration which is reported, along with the reference value’s uncertainty, in the manufacturer’s Certificate of Analysis (CoA) for the material. The reference material uncertainty is derived from its preparation by gravimetric and volumetric means and it includes the uncertainty in its analysis against a calibration curve generated using NIST certified reference materials. The gravimetric preparation of the reference material inherently contains uncertainty associated with the equipment used in the weighing, its calibration and the reference standards used in the process.

The CoA for the 0.100 g/100 mL ethanol control analyzed after each set of 5 simulator solution replicates is used to source this uncertainty. The CoA lists the relative (%), expanded uncertainty for the material (k=2, 95% confidence level). This is converted to the absolute, standard uncertainty through the following equation.

$$CV_{CoA}^2 = \left(\frac{u_{RM}}{1000 / 0.100} \right)^2$$

where:

CV_{CoA}^2 = the uncertainty in the value of the reference material

u_{RM} = the relative standard uncertainty of the reference material from the CoA

7.3.2 UNCERTAINTY FROM REPEATABILITY MEASUREMENTS (SIMULATOR SOLUTION)

Variations in repeated measurements of the simulator solution are derived under reproducibility conditions. These conditions consist of multiple factors including: the analyst, the headspace instrument calibration, instrument operation, environmental conditions, solution sampling and software calculations against the calibration curve. The variations in these results include uncertainty contributions from each of these factors.

This variability is represented through calculation of the relative standard deviation, or percent coefficient of variation, of the simulator solution concentration. First the average solution concentration is calculated using the following equation.

$$\bar{X} = \frac{1}{n} \sum_{i=1}^n X_i$$

where:

\bar{X} = the average simulator solution concentration

n = the number of measurements (e.g. 40 for external standard solutions, 15 for QAP solutions)

X_i = each individual simulator solution measurement result

i = incremental measurement results, first through last

The standard deviation (SD) of the simulator solution measurements is calculated using the following equation.

$$SD = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n - 1}}$$

The uncertainty from repeatability measurements of an external standard solution (CV_{ESS}^2) is calculated using the following equation.

$$CV_{ESS}^2 = \left(\frac{SD}{\bar{X}}\right)^2$$

The uncertainty from repeatability measurements of a QAP solution (CV_{QAP}^2) is calculated using the following equation. The standard deviation of the mean of 15 measurements is used in this equation.

$$CV_{QAP}^2 = \left(\frac{SD}{\sqrt{15}/\bar{X}}\right)^2$$

7.3.3 UNCERTAINTY FROM REPEATABILITY MEASUREMENTS (REFERENCE MATERIAL)

Variations in repeated measurements of the ethanol reference material are produced from the same uncertainty sources described in 18.3.2. The uncertainty for reference material repeatability ($CV_{Control}^2$) is calculated for external standard solution certification using the following equation.

$$CV_{Control}^2 = \left(\frac{SD}{\bar{X}}\right)^2$$

The uncertainty for the reference material repeatability ($CV_{Control}^2$) is calculated for QAP solution certification using the following equation.

$$CV_{Control}^2 = \left(\frac{SD}{\sqrt{3}/\bar{X}}\right)^2$$

7.3.4 UNCERTAINTY FROM INEXACT VALUES OF CONSTANTS

The uncertainty associated with the constant used to convert ethanol solution concentrations (g/100 mL) to ethanol vapor concentrations (g/210 L) is determined from fitting data to the exponential model describing the relationship between the water/air partition coefficient and temperature. The uncertainty for this constant ($CV_{Part\ Coef}^2$) is calculated using the following equation.

$$CV_{Part\ Coef}^2 = \left(\frac{0.0124}{1.23}\right)^2$$

7.3.5 COMBINED UNCERTAINTY FOR THE EXTERNAL STANDARD SOLUTION

The combined standard uncertainty of the external standard solution (u_{ESS}) is calculated using the following equation.

$$u_{ESS} = EVC \times \sqrt{CV_{CoA}^2 + CV_{ESS}^2 + CV_{Control}^2 + CV_{Part\ Coef}^2}$$

where:

EVC = equivalent vapor concentration of the solution

This calculation is done using the spreadsheet application, ESS Test Report Calculation Record. The expanded uncertainty for the external standard solution is obtained through multiplication by a coverage factor (k) of 2 which is equivalent to a 95% confidence level. The expanded uncertainty of the external standard solution is reported on the External Standard Solution Test Report with this confidence level.

7.3.6 COMBINED UNCERTAINTY FOR THE QAP SOLUTION

The combined standard uncertainty of a QAP solution (u_{QAP}) is calculated using the following equation.

$$u_{QAP} = EVC \times \sqrt{CV_{CoA}^2 + CV_{QAP}^2 + CV_{Control}^2 + CV_{Part\ Coef}^2}$$

This calculation is done using the spreadsheet application, QAP Test Report Calculation Record. An expanded uncertainty is not reported for the QAP solution and as such the uncertainty on this record can be understood to have a coverage factor (k) of 1. The QAP solution uncertainty is left as the combined standard uncertainty to facilitate its use directly in uncertainty calculations related to the instrument calibration and for use in any subsequent breath alcohol test uncertainty calculations. The QAP solution uncertainty is reported on the Quality Assurance Procedure Solution Test Report.

8 LIST OF CHANGES

Since Revision 3 (12/10/10)

Revision Date	Procedure	Change	Page Number
06/13/11	Overall format	Reformatted header & footer. Adopted new pagination for revisions to individual chapters. Included Chapter 19, List of Changes, to track chapter revisions. Total page count now appears in footer of Chapter 19.	All
06/13/11	Chapter 2	Procedure now allows the use of different volume flasks for preparation.	2-1 to 2-2
06/13/11	Chapter 5	Added second paragraph to include language about "as found" test.	5-2
06/13/11	Chapter 5	Added section 3.H.4 describing the inclusion of combined standard uncertainty on the Calibration Certificate.	5-6
06/13/11	Chapter 5 & 18	Removed reference to Datamaster Uncertainty Calculation Record (retirement).	5-6 to 5-7 & 18-6
06/13/11	Chapter 5	Added language to section 5.5.2 dealing with daylight savings time setting.	5-8
6/13/11	Chapter 17	Added language to 17.1 requiring BTT's to perform PT's on instruments they have personally calibrated.	17-1
10/10/11	Chapter 4	Modified balance description and GC column descriptions. Referenced separate ethanol calibrator procedure.	4-1, 4-2
10/15/12	Chapter 5, 6, 8-16	Deleted Chapter 5, 6, 8-16 and other policies and/or procedures pertaining only to the BTP.	All
10/15/12	Overall format	Document ID changed to TLDCalTM	All