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1.0 Purpose and Scope

1.1. The purpose of this procedure is to describe the process used by Vermont Forensic Laboratory (VFL) staff for the creation and certification of Certified Reference Materials (CRM), specifically wet bath simulator solutions for use with the DMT infrared breath alcohol analysis instruments.

1.2. The scope of this manual includes preparation of the simulator solutions, preparation of vials for analysis, instrument set-up, data review and release, documentation, and quality control criteria.

2.0 Responsibility

2.1. All analysts authorized to prepare and certify CRMs are responsible for following these procedures as written.

2.2. These procedures are reviewed periodically by the Toxicology Section staff. Revisions are made at that time or when there is an identified need to change this written manual to be compatible with changing needs in the analytical process. In the event that there are changes to be made to this manual, the changes must be reported in detail to the Toxicology Section Supervisor in a timely manner.

2.3. All analysts performing these procedures and reporting analytical results for forensic purposes must be fully trained and demonstrate initial competency in the use of these procedures in accordance with the Alcohol Training Manual (TOX_P300). All analysts will demonstrate ongoing competency by successfully completing one in-house practical test annually.

2.3.1. In-house practical tests consist of the preparation and certification of a solution which meets all the QA/QC requirements set forth in this manual.

2.4. Analysts will ensure that an adequate amount of supplies are on hand at all times. Orders should be placed when supplies are low to ensure that new stock arrives before supplies are completely empty.

3.0 Quality Assurance

3.1. It is expected that the analyst will report any unacceptable or anomalous behavior of any analytical system immediately to the Toxicology Section Supervisor. It is further expected that appropriate actions will follow as soon as possible and be properly documented.

3.2. Equipment

3.2.1. Balance

3.2.1.1. The analytical balance will be checked monthly with NIST traceable weights. This check will be recorded in the corresponding VFL Balances Equipment QA/QC Binder.

3.2.1.2. The analytical balance will have its calibration evaluated and certified by an approved vendor. The resulting documentation will be maintained.

3.2.1.3. Any balance maintained to the same standard may be used as a back-up if the primary balance is unavailable.

3.2.2. Pipettes

3.2.2.1. Measurements made by the Toxicology Section using pipettes are critical.

3.2.2.2. Pipettes used by the Toxicology Section, including fixed and variable volume pipettes have their calibration evaluated and certified by an approved vendor. The resulting documentation will be maintained.

3.2.2.3. If a pipette is sent out for service, an inspection of the package/pipette will be performed to check for any shipping and handling concerns prior to being returned for use. The calibration certificate will be reviewed in accordance with QA_P100_6.4 Equipment QC.

3.2.2.4. If a question arises regarding the proper functioning of a pipette, a performance check or calibration service by an approved vendor may be initiated.

3.2.2.4.1. Performance checks will be performed in accordance with QA_P100_6.4_Equipment QC.

3.2.2.4.2. Performance checks will be reviewed and filed in the VFL Pipettes Equipment QA/QC Binder.

3.3. Instrumentation

3.3.1. All maintenance performed, including routine and preventative maintenance as well as troubleshooting activities, should be recorded in the Instrument Maintenance Log.

3.3.2. Day of use

3.3.2.1. Ensure that the helium carrier gas is turned on with an appropriate delivery pressure (approximately 80 psi). Replace the cylinder if the remaining pressure in the tank is insufficient for analysis.

3.3.2.2. Ensure that the air compressor and zero air generator are turned on with an appropriate delivery pressure (approximately 45 psi).

3.3.2.3. Ensure that the hydrogen generator is turned on and that the deionized water reservoir is sufficiently full. If not, add deionized water to the reservoir.

3.3.2.4. See TOX_P100 for additional routine and annual maintenance procedures.

3.3.3. All equipment and materials, including the instrument maintenance log, are located in room 265 unless otherwise stated.

3.3.3.1. Perkin Elmer Clarus 580 gas chromatograph with flame ionization detector and Elite BAC-1 and BAC-2 Advantage columns, or equivalent.

3.3.3.2. Perkin Elmer TurboMatrix 110 headspace autosampler.

3.3.3.3. Desktop PC and printer with TotalChrom Workstation Software.

3.3.3.4. Parker Dominick Hunter hydrogen generator, or equivalent hydrogen supply.

3.3.3.5. Compressed UHP grade helium.

3.3.3.6. Jun Air compressor and Parker Balston Zero Air generator, or equivalent air supply.

3.4. Reference Materials

3.4.1. Calibration Standards

3.4.1.1. NIST traceable aqueous ethanol standards are used for calibration. Calibration standards with concentrations of 0.010, 0.020, 0.050, 0.080, 0.200, and 0.500 g/100 mL are purchased from an ISO/IEC 17025 and/or ISO 17034 accredited supplier.

3.4.1.2. Prior to using a new lot of calibration standard, one vial from the lot should be run as a sample (in duplicate) to verify the lot falls within ± 5% of the manufacturer's certified concentration. A new shipment of the same lot does not require verification.

3.4.1.3. After a new lot of calibration standard is verified, the data package from the verification run will undergo a Technical Review and be kept on file with the Toxicology Section. Completed verification packages should include the manufacturer's Certificate of Analysis (COA), calculation summary sheet, and all documents generated during the analysis as described in Section 6.2.

3.4.1.4. Calibration standards will be stored in the refrigerator.

3.4.2. Aqueous Ethanol Control

3.4.2.1. A NIST traceable aqueous ethanol standard is used for a within-run control in all analytical batches. An aqueous ethanol standard with a concentration of 0.080 g/100mL (of a different lot than the calibration standard) is purchased from an ISO/IEC 17025 and/or ISO 17034 accredited supplier.

3.4.2.2. Prior to using a new lot of aqueous ethanol standard, one vial from the lot should be run as a sample (in duplicate) to verify the lot falls within ± 5% of the manufacturer's certified concentration. A new shipment of the same lot does not require verification.

3.4.2.3. After a new lot of aqueous ethanol standard is verified, the data package from the verification run will undergo a Technical Review and be kept on file with the

Toxicology Section. Completed verification packages should include the manufacturer's COA, calculation summary sheet, and all documents generated during the analysis as described in Section 6.2.

3.4.2.4. Aqueous ethanol standards are stored in the refrigerator.

3.4.3. Verifications of reference materials will be documented in the Reagent Preparation Log.

3.4.4. Results from each analysis of calibration check samples and aqueous ethanol controls will be documented in the GC/FID Control Chart.

3.5. In-House Preparations

3.5.1.1. Solutions prepared in-house for use in CRM certification will be performance checked prior to use in casework. Specifications for these checks are defined in Section 5.0 of TOX_P100.

4.0 Simulator Solution Preparation

4.1. Reagents

4.1.1. Ethanol (200 proof ACS/USP grade)

4.1.2. Acetone (ACS reagent grade)

4.1.3. Methanol (ACS reagent grade)

4.1.4. Isopropanol (ACS reagent grade)

4.1.5. Copper sulfate ($CuSO_4$) (ACS reagent grade)

4.1.6. diH_2O

4.2. Apparatus

4.2.1. Analytical balance

4.2.2. Pipettes or syringes for aliquoting solutions

4.2.3. Volumetric flasks

4.2.4. Plastic carboy with spigot (20 L)

4.2.5. Erlenmeyer flasks

4.2.6. Plastic round bottles (500 ml)

4.2.7. Parafilm or seals

4.2.8. Induction sealing machine

4.2.9. Easy peel white mailing labels (size 1" x 2 5/8")

4.2.10. Adhesive plastic sleeves

4.3. Interference Solution Preparation

4.3.1. Measure desired volume of stock solution in a volumetric flask (certified ethanol solution or diH_2O).

4.3.2. Add correct volume of interference material (acetone, methanol or isopropanol) to achieve desired concentration, measured by volume (e.g. 200 µl acetone added to 2 L of 0.08 g/210 L ethanol solution yields a final concentration of 0.01% vol/vol acetone in 0.08 g/210 L ethanol).

4.3.3. Assign a simulator solution lot number for each solution using the scheme YY-NN-XXIX; where:

4.3.3.1. YY = The last two digits of the year in which the solution was made;

4.3.3.2. NN = The next sequential solution number for the year.

4.3.3.3. XX = The target ethanol concentration expressed to two decimal places.

4.3.3.4. IX = The interferent present in the solution followed by the concentration.

4.3.3.5. E.g. 22-01-08A2 where 22 is the year, 01 is the first solution prepared that year, 08 refers to 0.080 g/210 L ethanol, A refers to Acetone, and 2 refers to 0.02% vol/vol acetone concentration in the solution.

4.3.4. Record all solution information in the Reagent Preparation Log.

4.3.5. One vial of a newly prepared interference solution is analyzed via GC/FID and deemed acceptable for use when each compound in the mixture is detected with baseline separation and the retention times are consistent with the components of the mixture.

4.3.6. Create an Interference Solution Certificate of Analysis (TOX_F500_1) and lot number labels (See Appendix A for label example).

4.3.7. Solutions prepared using a previously certified ethanol solution may already contain cupric sulfate. If not, all solutions must have approximately 0.25 g cupric sulfate per 2 L of solution added prior to bottling.

4.3.8. Transfer the solution to appropriately labeled 500 ml screw-cap, narrow mouth, chemical resistant bottles and seal.

4.3.9. Interference solution bottles will receive three lot number labels. One label will be affixed to the bottle, and the other labels will be placed in the plastic sleeve, which is attached to the bottle.

4.3.10. The chromatograms from the analysis will be reviewed and documentation of passing QC recorded in the Reagent Preparation Log. The Interference Solution Certificate of Analysis, Reagent Preparation Log and labels are reviewed and approved by the

Toxicology Section Supervisor or their designee prior to use. Analytical results will be kept on file with the Toxicology Section.

4.3.11. Solutions expire two years from date of parent solution preparation.

4.3.12. Solutions should be stored in a controlled environment at room temperature.

4.4. Calibration and Certification Solution Preparation

4.4.1. The following table describes the mass of absolute ethanol required to prepare solutions which will provide the listed equivalent breath alcohol concentrations [BrAC] when equilibrated in a breath-alcohol simulator at 34.0ºC:

Equivalent BrAC	Mass of Absolute Ethanol	Final Volume
0.02 g/210 L	0.4920 g	2 L
0.04 g/210 L	0.9840 g	2 L
0.08 g/210 L	4.9200 g	5 L
0.10 g/210 L	6.1500 g	5 L
0.16 g/210 L	3.9360 g	2 L
0.36 g/210 L	8.8560 g	2 L

4.4.2. When making a solution of a different final volume from the above chart, the following equation can be used to determine the mass of absolute ethanol needed to make the desired final volume:

$$\text{Mass of Absolute Ethanol [g]} = \frac{\text{(Final Volume [L] x Equivalent BrAC)}}{\text{(0.1[L] / 1.23*)}}$$

*Partition coefficient for aqueous ethanol to headspace ethanol at 34°C (See Section 9.1)

4.4.3. Gravimetrically dispense the appropriate amount of absolute ethanol into glassware which is partially filled with diH_2O.

4.4.4. Transfer the ethanol/water mixture into the desired size volumetric flask and fill to volume with diH_2O. Invert to mix thoroughly.

4.4.5. Assign a simulator solution lot number for each solution using the scheme YY-NN-XXX; where:

4.4.5.1. YY = The last two digits of the year in which the solution was made;

4.4.5.2. NN = The next sequential solution number for the year.

4.4.5.3. XXX = The target concentration expressed to three decimal places (ex. a concentration of 0.080 would be expressed as 080; a concentration of 0.160 would be expressed as 160).

4.4.6. Record all solution information in the Reagent Preparation Log.

4.4.7. Solution expires two years from date of preparation.

4.4.8. Solutions should be stored in a controlled environment at room temperature.

4.4.9. Analyze and certify the solution as described in Sections 5.0 and 6.0.

4.4.10. After the solution is certified and documentation reviewed, add approximately 0.25 g of cupric sulfate per 2 L of solution to the flask and swirl until all crystals are dissolved.

4.4.11. Transfer the solution to appropriately labeled 500 ml screw-cap, narrow mouth, chemical resistant bottles and seal.

4.4.12. These bottles will receive three lot number labels (See Appendix A for example). One label will be affixed to the bottle, and the other two labels will be placed in the plastic card holder, which is attached to the bottle.

4.5. External Standard Solution Sample Preparation

4.5.1. A simulator solution of 0.10 g/210 L is used as a quality control sample on DMT instruments in the field. 20 L batches are made and certified at the VFL and are distributed to DMT agencies for field use.

4.5.2. Weigh approximately 24.6 g of absolute ethanol into a flask, which is partially filled with diH_2O.

4.5.3. Fill the 20 L plastic carboy using four additions of diH_2O from a 5 L class A volumetric flask, adding the ethanol/water mixture to the second 5 L addition of diH_2O. Shake well and allow solution to sit at least overnight.

4.5.4. Assign a simulator solution lot number as described in Section 4.4.5.

4.5.5. Record all solution information in the Reagent Preparation Log.

4.5.6. Solution expires two years from date of preparation.

4.5.7. Solutions should be stored in a controlled environment at room temperature.

4.5.8. Analyze and certify the solution as described in Sections 5.0 and 6.0.

4.5.9. After the solution is certified and documentation reviewed, add approximately 2.5 g of cupric sulfate to it. Mix the carboy until the crystals have dissolved.

4.5.10. Transfer the solution to appropriately labeled 500 ml screw-cap, narrow mouth, chemical resistant bottles and seal.

4.5.11. These bottles will receive three lot number labels (See Appendix A for example). One label will be affixed to the bottle, and the other two labels will be placed in the plastic sleeve attached to the bottle.

4.5.12. At least one bottle from each batch will be stored in a controlled environment at room temperature for the lifetime of the lot.

5.0 Headspace GC/FID Analysis of Ethanol Simulator Solutions

5.1. Ethanol simulator solution analysis will be performed by two different analysts. The analytical batch will be run against the calibration curve prepared by that analyst.

5.2. Prior to sampling a 20 L batch, rinse the spigot with fresh solution by pouring off and discarding a small amount of simulator solution.

5.3. Each analyst will pour off a small aliquot of simulator solution from the spigot when analyzing the 20 L batch on the day of analysis. This aliquot will be used to prepare sample vials.

5.4. See TOX_P100 for sample preparation criteria and instrument setup procedures.

5.5. Label one 20 mL round bottom headspace autosampler vial for each of the following:

5.5.1. Opening aqueous blank.

5.5.2. Timing Mix.

5.5.3. Each calibration level, A through E and G.

5.5.4. Aqueous control in duplicate.

5.5.5. A sufficient number of Calibration Check Samples (CCS).

5.5.5.1. CCS will consist of 0.050 g/100 mL (low) and 0.200 g/100 mL (high) calibration standards.

5.5.5.2. CCS will be analyzed in duplicate after QC and at the end of each analytical batch, with no more than 10 sample vials bracketed between CCS vials. Low and high concentration CCS will alternate throughout the batch.

5.5.6. A set of 10 vials containing simulator solution reference material.

5.5.7. Closing aqueous blank.

5.6. Simulator solution samples will be divided by a partition coefficient of 1.23 to convert aqueous ethanol concentration to equivalent concentration of ethanol in 210 L of air at 34˚C. Instrumental methods are defined in the Instrument Maintenance Log.

6.0 Solution Certification and Review

6.1. Upon successful completion of analyses, the results from both runs will be compiled using the Simulator Solution Worksheet (TOX_F500_2). The analyst must perform a primary data

review of the package prior to submitting the complete package to the assigned reviewer for technical review.

6.2. The completed package includes:

6.2.1. Certified Reference Material Review Checklist (QA_F100_7.7_14).

6.2.2. Certificate of Analysis (TOX_F500_3).

6.2.3. Simulator solution lot number labels.

6.2.4. QC Summary, IS Area Count worksheet, and CRM worksheet from the Simulator Solution Worksheet (TOX_F500_2).

6.2.5. Batch sequence lists.

6.2.6. Instrument calibration graphs.

6.2.7. All chromatograms generated during the analytical process.

6.3. Analyst Review

6.3.1. Aqueous blanks, timing mix, aqueous controls, and calibration check samples should adhere to quality control criteria as outlined in TOX_P100.

6.3.2. The six certified ethanol calibration standards should fall within 10% of the following concentrations when rounded to 3 decimal places:

STD A	0.010 g/100 ml
STD B	0.020 g/100 ml
STD C	0.050 g/100 ml
STD D	0.080 g/100 ml
STD E	0.200 g/100 ml
STD G	0.500 g/100 ml

6.3.3. The correlation coefficient of the calibration line for each channel must be 0.99 or greater. If not, the calibration must be repeated.

6.3.4. Simulator solutions not meeting quality control criteria may be reanalyzed provided that they are accompanied by bracketing aqueous blanks and CCS, the same internal standard lot number is used, and the samples are run within 24 hours of the original calibration.

6.3.5. Ten analytical vials of simulator solution should meet the following criteria:

6.3.5.1. All analysis results must fall within 5% of the mean of all replicates, rounded to four decimal places.

6.3.5.2. All analysis results must fall within 3 standard deviations of the mean of all

replicates, rounded to four decimal places.

6.3.5.3. All analysis results must yield a coefficient of variation below 5%, calculated as:

$$\%CV = \frac{s}{mean\ response}$$

Where *s* is the standard deviation of all channel results for the analytical batch and the mean response is the average of all analysis results, rounded to four decimal places.

6.3.6. Surrogate compound concentrations for each sample are between 0.900 and 1.100

6.3.7. Internal standard peaks for quantitative samples must fall within ±20% of the average internal standard peak area from the current calibrators.

6.3.8. Ensure that all samples are quantified against the correct calibration curve.

6.3.9. Replicate results from both batches should meet the following criteria:

6.3.9.1. All analysis results must fall within 5% of the grand mean of all replicates, rounded to four decimal places.

6.3.9.2. All analysis results must fall within 3 standard deviations of the grand mean of all replicates, rounded to four decimal places.

6.3.9.3. All analysis results must yield a coefficient of variation below 5%, calculated as in Section 6.3.5.3.

6.3.9.4. Reported solution concentration will be rounded to three decimal places before measurement uncertainty is calculated, as outlined in Section 7.0.

6.3.9.5. Calibration and External Standard solutions must have a reported concentration within 3% of the target value to be acceptable. All other solutions must have a reported concentration within 10% of the target value.

6.3.10. Individual outlying replicates may be omitted on a case by case basis with approval from the Toxicology Section Supervisor.

6.4. Technical Review:

6.4.1. The assigned reviewer must perform a technical review of the complete data package.

6.4.2. Ensure that forms are complete and accurate. The reviewer will confirm that the information on the storage bottle labels (lot #, concentration, prepared date) matches the information in the Reagent Preparation Log.

6.4.3. Confirm all calculations that are not part of a validated worksheet.

6.4.4. If data quality issues have been identified during data review, the reviewer must attempt resolution through discussion with the analyst and/or section supervisor. If issues cannot be resolved, it may be necessary to repeat the analytical procedure.

6.4.5. Solutions not meeting acceptance criteria will be discarded.

6.5. Upon completion of the technical review, an administrative and director review of the data package will be completed.

7.0 Estimation of Uncertainty of Measurement

7.1. The estimation of measurement uncertainty is performed using the GUM Approach as defined in the ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – Annex A.

7.1.1. A 99.73% level of confidence will be used to determine the expanded uncertainty.

7.1.2. The expanded uncertainty will be rounded up to two significant figures.

7.2. Calculate the confidence interval for each result by multiplying the measured result by the expanded uncertainty. This value will be reported along with the measured result.

7.2.1. The reported estimated measurement uncertainty will be truncated to four decimal places, then rounded up to three decimal places.

7.3. To assist with the application of simulator solution results, the interval will be reported in the following format:

$$0.XXX \pm 0.YYY \text{ g/210 L ethanol}$$

7.4. The estimated uncertainty of measurement will be reviewed at least annually or if any significant change in the expanded uncertainty is suspected.

7.5. The reported result is the result at the time of analysis, and does not account for changes in sample composition which may occur subsequent to or before examination.

8.0 Abbreviations

8.1. CRM = Certified Reference Material

8.2. CV = Coefficient of Variation

8.3. GC/FID = Gas Chromatograph / Flame Ionization Detector

8.4. COA = Certificate of Analysis

8.5. BrAC = Breath Alcohol Concentration

8.6. STD = Standard

8.7. IS = Internal Standard

8.8. CCS = Calibration Check Sample

8.9. GUM = Guide to the Expression of Uncertainty in Measurement

9.0 References

9.1. Hwang, R., Beltran, J., Rogers, C., Barlow, J., & Razatos, G.; "Measurement of Uncertainty for Aqueous Ethanol Wet-Bath Simulator Solutions Used with Evidential Breath Testing Instruments", Journal of Forensic Sciences, Vol. 61, No. 5, Sept. 2016, 1359-1363.

9.2. Dubowski, K.M.; "Storage Stability of Simulator Ethanol Solutions for Vapor-Alcohol Control Tests in Breath Alcohol Analysis", Journal of Analytical Toxicology, Vol. 26, Oct. 2002, 406-410.

9.3. Alcohol Training Manual (TOX_P300)

9.4. VFL Balances Equipment QA/QC Binder

9.5. VFL Pipettes Equipment QA/QC Binder

9.6. Toxicology Section Reagent Preparation Log

9.7. Interference Solution Certificate of Analysis (TOX_F500_1)

9.8. Alcohol Analysis Manual (TOX_P100)

9.9. Simulator Solution Worksheet (TOX_F500_2)

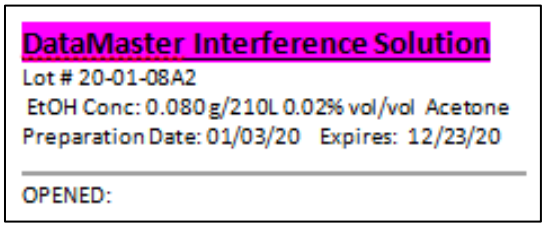
9.10. Certified Reference Material Review Checklist (QA_F100_7.7_14)

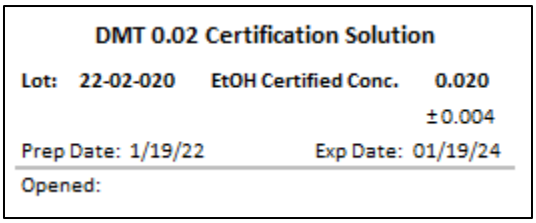
9.11. Certificate of Analysis (TOX_F500_3)

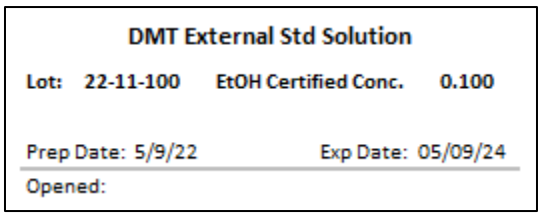
9.12. ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – Annex A; Details on the NIST 8-Step Process. ASCLD/LAB – International.

Appendix A

Label Examples:







DOCUMENT HISTORY			
DATE	**VERSION**	**APPROVED BY**	**ACTIVITY OR REVISION**
10/18/2016	1	Lab Director	Manual updated to reflect new instrumentation and procedure; ALC_P500 replaces ALC_P201; ALC_F500_1 replaces ALC_F201_1; ALC_F500_2 replaces ALC_F201_2; QA_F100_5.9_14 replaces ALC_F201_3; ALC_F500_3 replaces ALC_F201_4
10/25/2016	2	Lab Director	Appendix A added
11/5/2018	3	Lab Director	Changed all "ALC" references to "TOX" and updated manual numbers; updated sections 2.3.1 (competency), 3.2.1.5 (back up balance) and 3.2.2 (pipettes); minor formatting changes throughout; updated TOX_F500_1 (header & form number), TOX_F500_2 (form number & formula fix to allow more than one set of CCSs) and TOX_F500_3 (header & form number, removed reference to balance)
6/24/2020	4	Lab Director	Removed daily balance checks, aligned pipette QC language with equipment QC manual, updated expiry date of solutions to two years, updated label examples, referenced TOX_P100 for QC criteria requirements, added Table of Contents, minor formatting changes throughout document
8/10/2022	5	Lab Director	Updated equipment QC section, removed historical calibrations, minor formatting changes throughout; TOX_F500_2 updated to only use calibrators, not blank, for IS average calculation