

# VERMONT FORENSIC LABORATORY

## Certified Reference Material Training Manual

Doc. No.  
TOX\_P305\_Version 1

Approved by:  
Lab Director

Effective Date:  
07182024  
Status: Active

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### 1.0 Training Overview

#### 1.1. Training Purpose and Description

##### 1.1.1. Purpose and Goals

The purpose of this training program is to provide a uniform training process for analysts in the Toxicology Section at the Vermont Forensic Laboratory (VFL). This program is designed to ensure and document that those individuals who will be working as analysts are knowledgeable and competent to perform their technical, analytical, and legal duties.

##### 1.1.2. Scope

This training manual should be completed in conjunction with the TOX\_P300\_Intro to Toxicology Training Manual. Training will concentrate on Certified Reference Material (CRM) analysis methods currently in use at the VFL and will culminate in a competency test(s). This program is designed for new employees or current employees without prior certified reference material analysis experience. A trainee with previous experience in forensic or other toxicological analysis may not require all modules or steps; it is the responsibility of the Toxicology Section Supervisor to determine the duration and scope of the training program for a trainee with previous experience. Similarly, the module content may be tailored as applicable to anticipated job responsibilities.

##### 1.1.3. Documentation

The trainee will compile all documentation associated with training work completed. These files may include, but are not limited to, worksheets, reports, and review sheets. The trainer will review these materials and document completion of required training components. Documentation of training will be maintained at the laboratory.

### 1.2. Trainee Responsibilities

#### 1.2.1. Instructions for Trainee

The length of time needed to complete the training program will vary and is left to the discretion of the trainer and supervisor. The trainee will be provided access to any required or suggested readings and will be exposed to samples and situations expected to be encountered during routine work in the Toxicology Section. The trainee will keep records, where appropriate, of how training tasks were accomplished (e.g. what additional papers not listed in Appendix I did the trainee reference, etc.). At the conclusion of training, the trainee will evaluate the effectiveness of the training program and suggest any improvements to the section supervisor.

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### 1.2.2. Required Training Modules

The trainee, trainer, and section supervisor shall discuss which portions of the training manual are to be completed by the trainee based on the trainee’s anticipated job responsibilities and prior experience. This section may also be used to outline re-training requirements for current employees if needed. The requirements for the trainee are outlined below:

	Required?		Completed?	
	Yes	No	Date	Trainer
1. Training Overview	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
2. Laboratory Introduction				
3. Sample and Evidence Control				
4. Fundamental Scientific Knowledge				
5. Applied Scientific Knowledge				
5.1 Gas Chromatography Theory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
5.2 CRM Analysis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
5.3 Statistics and Data Analysis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
6. Laboratory Analysis				
6.1 CRM Analysis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
6.2 Training Sample Processing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
7. Reports and Notifications				
7.1 Report Writing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
7.2 Review	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
7.3 Mock Case/Practical Scenario	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
8. Legal Knowledge				
9. Final Evaluation				
9.1 Competency Tests	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
10. Authorization	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>

### 1.3. Trainer Responsibilities

The trainer is responsible for instructing the trainee in the operations of the laboratory and the processes and procedures that will ultimately comprise the trainee’s job duties. The trainer will ensure that the trainee is exposed to all relevant topics within the training program. The trainer will request various solutions for the trainee to prepare and analyze and will meet with the trainee periodically to monitor progress, review work, and provide feedback. The trainer will assist the

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trainee in preparing for any assessments, which will include a competency test. At the conclusion of training, the trainer will evaluate the effectiveness of the training program and suggest any improvements to the section supervisor.

### 1.4. Acknowledgement of Training Plan

The signatures of the trainee, trainer(s), and section supervisor below indicate that the expected responsibilities and required training modules have been discussed and agreed upon.

Trainee: \_\_\_\_\_ Date: \_\_\_\_\_

Trainer(s): \_\_\_\_\_ Date: \_\_\_\_\_

Trainer(s): \_\_\_\_\_ Date: \_\_\_\_\_

Section Supervisor: \_\_\_\_\_ Date: \_\_\_\_\_

### 2.0 Laboratory Introduction

Refer to TOX\_P300\_Intro to Toxicology Training Manual.

### 3.0 Sample and Evidence Control

Refer to TOX\_P300\_Intro to Toxicology Training Manual.

### 4.0 Fundamental Scientific Knowledge

Refer to TOX\_P300\_Intro to Toxicology Training Manual.

### 5.0 Applied Scientific Knowledge

This training module will ensure that the trainee has received appropriate education and training to apply principles of gas chromatography and statistics to the analysis of CRMs.

#### 5.1. Gas Chromatography Theory

The trainee will develop and demonstrate a working knowledge of the techniques in use at the VFL to analyze aqueous based alcohol solutions, including the use of headspace gas chromatography (GC) with dual flame ionization detectors for certified reference material analysis.

Task	Trainee	Trainer	Date Completed
I have reviewed a certification packet for the certification of simulator solutions by gas chromatography.			

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I have read and understand the required readings outlined for this section in Appendix I.			
I have answered the questions outlined for this section in Appendix II and received feedback on my answers.			

### 5.2. CRM Analysis

The trainee will develop and demonstrate an understanding of the instrumentation and procedures used for CRM preparation and analysis.

<b>Task</b>	<b>Trainee</b>	<b>Trainer</b>	<b>Date Completed</b>
I have read and understand the required readings outlined for this section in Appendix I.			
I have answered the questions outlined for this section in Appendix II and received feedback on my answers.			

### 5.3. Statistics and Data Analysis

The trainee will develop and demonstrate a working knowledge of the quantitative reporting, statistical calculations, and measurement uncertainty factors applied to CRM analysis in use at the VFL.

<b>Task</b>	<b>Trainee</b>	<b>Trainer</b>	<b>Date Completed</b>
I have reviewed the measurement uncertainty budgets for CRM analysis.			
I have read and understand the required readings outlined for this section in Appendix I.			
I have answered the questions outlined for this section in Appendix II and received feedback on my answers.			

### 6.0 Laboratory Analysis

The trainee will demonstrate the ability to apply knowledge of the currently validated methods and technologies to the analysis of training solutions representing various concentrations of certified reference materials produced at the VFL.

#### 6.1. CRM Analysis

The trainee will develop and demonstrate a working knowledge of the instrumentation and procedures used for CRM analysis, including reagent preparation, instrument operation, quality

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control and maintenance of the gas chromatograph and headspace autosampler. This section may be completed concurrently with TOX\_P301. The trainee and trainer will document this by both initialing the boxes below.

Task/Procedure	Initials & Date Observed	Initials & Date Performed
Preparation of reagents for CRM analysis (including use of the Alcohol Reagent Log)		
General use of the gas chromatograph and headspace autosampler		
Instrument maintenance and record keeping (Maintenance Log)		
I have read and understand the required readings outlined for this section in Appendix I.		
I have answered the questions outlined for this section in Appendix II and received feedback on my answers.		
I have been authorized to use the headspace GC-FID.		

### 6.2. Training Sample Processing

The trainee will apply theoretical knowledge of the protocols and procedures in use at the VFL to the preparation and certification of simulator solutions by GC. Prior to preparing and certifying a training simulator solution, the trainee will observe a qualified analyst(s) prepare and certify a simulator solution. The trainer will request various solutions for the trainee to prepare and analyze. The trainee will document each analysis performed and provide the trainer with results from the analyses. The trainee will demonstrate an understanding of the limitations and sensitivity of the procedure and associated quality controls. The trainee and trainer will document this by both initialing the boxes below.

Task/Procedure	Initials & Date Observed	Initials & Date Performed Under Supervision	Initials & Date Performed Independently
Solution Preparation			
GC Analysis			

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### 7.0 Reports and Notifications

The trainee will develop and demonstrate the ability to generate data packages and certificates of analysis (COA) created during the certification of simulator solutions.

#### 7.1. Report Writing

The trainee will develop and demonstrate knowledge of procedures and documentation requirements for preparing and certifying reference materials in accordance with laboratory policy. Training will include, but is not limited to, report format, language used, and the use of various logs used in tracking simulator solutions. The trainee and trainer will document this by both initialing the boxes below.

Task/Procedure	Initials & Date Observed	Initials & Date Performed Under Supervision	Initials & Date Performed Independently
Generated a CRM data package			
Generated a COA			
I have read and understand the required readings outlined for this section in Appendix I.			
I have answered the questions outlined for this section in Appendix II and received feedback on my answers.			

#### 7.2. Review

The analyst trainee will become familiar with the policies, procedures, and forms for technical, administrative, and director review of CRM data packages and certificates of analysis.

Task	Trainee	Trainer	Date Completed
I am familiar with the technical review requirements as outlined in the QA, Certified Reference Material, and Alcohol Analysis Manuals.			
I have observed the technical review of CRM data packages and COAs by an experienced analyst.			
I have performed “pre-review” technical review(s) of CRM data packages and COAs.			
I have observed the administrative/director review of CRM			

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data packages and COAs.

### 7.3. Mock Case/Practical Scenario

The trainer will devise at least one practical scenario for the trainee to complete. The practical scenario will include the preparation, analysis, certification, and bottling of a simulator solution as requested by the trainer. The acceptance criteria for this practical will be documented by the section supervisor prior to starting. The trainee will complete the required work and prepare the CRM package and COA for review. The trainer will perform reviews of these reports, check the bottles for proper seals, and provide feedback on the results as needed to meet review criteria.

Task	Trainee	Trainer	Date Completed
I have completed a practical scenario to include the preparation, analysis, certification, and bottling of a simulator solution.			

### 8.0 Legal Knowledge

The preparation and certification of simulator solutions does not generally involve routine testimony. Analyst training should be sufficient so that the analyst understands their role in the judicial system, but need not be more specific than the training received in the course of the TOX\_P300\_Intro to Toxicology Training Manual.

### 9.0 Final Evaluation

At the completion of this training program, the trainee's ability to perform the duties of an analyst will be assessed. The nature of final assessment and evaluation may differ based on the trainee's experience and anticipated job responsibilities. The section supervisor is responsible for determining what assessment and evaluation is necessary for the trainee and documenting this.

### 9.1. Competency Tests

The trainee will pass all applicable competency tests as determined and documented by the section supervisor prior to beginning work as an analyst. Competency testing includes a written exam and will require the preparation of a written report, which will undergo technical, administrative, and director reviews. Satisfactory completion of a competency test is required for all analysts regardless of previous experience. The nature of the competency test should be sufficient to cover the anticipated spectrum of assigned duties and to evaluate the individual's ability to perform proper testing methods.

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<b>Task</b>	<b>Trainee</b>	<b>Trainer</b>	<b>Date Completed</b>
I have completed all competency tests pertaining to CRM preparation, analysis, and certification.			
I have successfully completed a written examination pertaining to CRM analysis and received feedback on my results.			

### 10.0 Authorization

Completion of training modules will be documented in section 1.2.2. At the completion of training, the section supervisor shall provide written documentation authorizing the trainee to prepare and analyze certified reference materials as a qualified analyst.

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### Appendix I: Readings

#### A: Required Readings

The resources listed below are provided to give the trainee the necessary information to perform their duties. Depending on the trainee's education and experience they may not need to complete every reading in its entirety. The location of various training tools such as readings or websites are included for convenience. If file locations change or links break, alternative training materials may be used. Supplemental documents are available through the laboratory's shared drive under the training folder, but are not required unless designated as such by the section supervisor. The trainee should be familiar with the readings provided in the TOX\_P300 manual in order to assist them in answering the questions in Appendix II.

#### 5.0 Applied Scientific Knowledge

##### 5.1. Gas Chromatography Theory

- Tiscione, et al. "Ethanol Analysis by Headspace Gas Chromatography with Simultaneous Flame-Ionization and Mass Spectrometry Detection", Journal of Analytical Toxicology, Vol 35, 2011, pp. 501-511.
- Tipler, A.; "An Introduction to Headspace Sampling in Gas Chromatography – Fundamentals and Theory" Perkin Elmer reference guide

##### 5.2. CRM Analysis

- ASB Standard 055: Standard for Breath Alcohol Measuring Instrument Calibration DRAFT 2023.
- R.Sander, "Compilation of Henry's law constants (version 4.0) for water as solvent", Atmos. Chem. Phys., 15, p. 4399-4981, 2015
- VFL Alcohol Analysis Manual (TOX\_P100) and all associated documents
- VFL Certified Reference Material Manual (TOX\_P500) and all associated documents
- VFL CRM Method Validation
- Analytical Methods portion of the VFL Toxicology Reference Library

##### 5.3. Statistics and Data Analysis

- Simulator Solution Worksheet (TOX\_F500\_2)
- Current and historical CRM MU budgets
- CRM fishbone step 2 and step 3
- GC-FID control chart
- 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.

#### 6.0 Laboratory Analysis

##### 6.1. CRM Analysis

- Alcohol reagent log
- GC-FID instrument maintenance log

#### 7.0 Reports and Notifications

##### 7.1. Report Writing

- Interference Solution Certificate of Analysis (TOX\_F500\_1)

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- Simulator Solution Worksheet (TOX\_F500\_2)
- Certification of Analysis (TOX\_F500\_3)

### **B: VFL Toxicology Reference Library**

Analysts are expected to be familiar with the contents of the VFL Toxicology Reference Library and be alert for articles and/or references that can be added. Updated references should be added to the VFL Toxicology Reference Library during the review period, when they become available, or when new methodologies or technologies are incorporated into the laboratory protocols.

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### Appendix II: Training Questions

Questions listed below are intended to be answered by trainees. Headings are numbered in accordance with the section numbering in the body of this manual.

#### 5.0 Applied Scientific Knowledge

##### 5.1. Gas Chromatography Theory

1. Explain the principle and operation of headspace gas chromatography.
2. Explain the principle and operation of flame ionization detection.
3. What gases are used in GC-FID analysis and what are their purposes?
4. Explain the calibration process of the GC-FID.
5. Manually calculate an alcohol concentration based on response of ethanol, internal standard, and calibrators.
6. What is a surrogate? What is its role? What are the relevant QC criteria?
7. What is a timing mix? What is its role? What are the relevant QC criteria?

##### 5.2. CRM Analysis

1. What is a certified reference material?
2. What is NIST and what role does it play in the certification of simulator solutions?
3. Who prepares simulator solutions?
4. What pipetting method is used to aliquot solution? Internal standard?
5. What is the internal standard used for CRM analysis?
6. What are high and low aqueous controls? What are their roles? What are the relevant QC criteria?
7. Describe the process for certifying a simulator solution by GC.
8. Describe a typical run sequence for CRM analysis.
9. What is the VFL's calibration range for CRMs?

##### 5.3. Statistics and Data Analysis

###### Statistical Analysis

1. How many calibration points are required for the CRM curve based on the ASB guidelines?

###### Excel

1. How does Excel source the QC information from the raw data?
2. How does Excel determine if a replicate does not meet acceptance criteria?
3. What can you edit in the simulator solution worksheet (TOX\_F500\_2)?
4. How are CRM results rounded and truncated?
5. How is the data sourced for the CRM summary page from the raw data?
6. What must be manually typed into the spreadsheet vs automatically sourced?
7. How are results reported?

###### Measurement Uncertainty

1. How is the measurement uncertainty of the CRM method estimated?

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2. How often is the budget updated?
3. What is the timeframe of the data used in the budget?
4. What confidence interval is applied for CRMs?
5. What contributors are evaluated?
6. What are the homogeneity and stability measurements of?
7. Which uncertainty value do we input for the pipettes?

### 6.0 Laboratory Analysis

#### 6.1. CRM Analysis

1. What information should appear on the label of a reagent prepared in the laboratory?
2. How are reagents prepared in the laboratory QC checked? How are these QC checks documented?
3. What QC checks do purchased reagents require?
4. Who is responsible for ordering supplies for reagents prepared in-house? How does this process work?
5. What QC checks and maintenance does each of the following require?
  - a. Perkin Elmer TurboMatrix 110 HS autosampler
  - b. Currently validated GC-FID
  - c. Gas tanks
  - d. Hydrogen generator

### 7.0 Reports and Notifications

#### 7.1. Report Writing

1. What documents are included in CRM certification data packages?
2. What is the naming convention for simulator solutions?
3. What are the acceptance criteria for accepting and certifying a simulator solution?
4. What is the shelf life for simulator solutions?
5. What does gravimetric mean?
6. What is the conversion factor used when certifying a simulator solution?
7. Why is a conversion factor used?
8. What are the various uses for simulator solutions and how is this conveyed on a COA?
9. What information must be included on a CRM COA?

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### DOCUMENT HISTORY

DATE	VERSION	APPROVED BY	ACTIVITY OR REVISION
7/18/2024	1	Lab Director	New document; content previously part of ALC P300