EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1274

LOW-VOLATILITY AGENT PERMEATION (LVAP) VERIFICATION AND VALIDATION REPORT

Terrence G. D'Onofrio
John P. Davies
RESEARCH AND TECHNOLOGY DIRECTORATE

Christopher B. Steinbach
EXCET, INC.
Springfield, VA 22151-2110

Christopher J. Ruppert
ENGINEERING DIRECTORATE

May 2015

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-05-2015	Final	Jan 2014 – Sep 2014

4. TITLE AND SUBTITLE
Low-Volatility Agent Permeation (LVAP) Verification and Validation Report

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
D'Onofrio, Terrence G.; Davies, John P. (ECBC); Steinbach Christopher B. (EXCET); Ruppert, Christopher J. (ECBC)

5d. PROJECT NUMBER
HDTRA1411111

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRT-O//RDCB-DET-A, APG, MD 21010-5424
EXCET, Inc., 8001 Braddock Road, Suite 303, Springfield, VA 22151-2110

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1274

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Deputy Under Secretary of the Army for Test and Evaluation
102 Army Pentagon
Washington, DC 20310-0102

10. SPONSOR/MONITOR'S ACRONYM(S)
DUSA-TE

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
This report provides specific details for the verification and validation (V&V) of a low-volatility agent permeation (LVAP) test methodology. Upon acceptance of this V&V report, this methodology will be transitioned to the Test and Evaluation (T&E) community for use in current and future acquisition programs. LVAP test methods have been shown to be more accurate for measuring the permeation of low-volatility contaminants such as *O*-ethyl *S*-[2-ethyl] methylphosphonothioate (VX). The traditional methods detailed in various standards using a liquid challenge and a vapor sample collection are problematic when applied to low-volatility compounds. The method results detailed in this report were derived from multiple years of research at the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) with support from the Joint Science and Technology Office (JSTO; Ft. Belvoir, VA); the U.S. Army Natick Soldier Research, Development, and Engineering Center (NSRDEC; Natick, MA); and the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD; Aberdeen Proving Ground, MD).

15. SUBJECT TERMS

Verification and validation (V&V)	Permeation	Personal protective equipment (PPE)
Low-volatility agent permeation (LVAP)	Extraction efficiency	Test development

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu B. Rastogi
U	U	U	UU	166	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

Blank

PREFACE

The work described in this report was authorized under the Office of The Deputy Under Secretary of the Army for Test and Evaluation (DUSA-TE). The work was started in January 2014 and completed in September 2014.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. Manufacturer names and model numbers are provided for completeness. This technical report may not be cited for purposes of advertisement.

This report has been approved for public release.

Acknowledgments

A program cannot be successfully completed without the contributions of a good team of people. Many stakeholders in the community provided comments to help make this verification testing successful. These stakeholders included representatives from U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD), West Desert Test Center (WDTC; Dugway Proving Ground, UT), Battelle Memorial Institute (Columbus, OH), Joint Project Manager for Protection (JPM P), Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD; Aberdeen Proving Ground, MD), National Institute of Standards and Technology (NIST; Gaithersburg, MD), U.S. Army Test and Evaluation Command (ATEC; Aberdeen Proving Ground, MD), U.S. Army Natick Soldier Research, Development, and Engineering Center (NSRDEC; Natick, MA), Marine Corps Operational Test and Evaluation Activity (MCOTEA; Quantico, VA), and Deputy Under Secretary of the Army for Test and Evaluation (DUSA-TE; Washington, DC).

The authors specifically thank Megan Holste (DUSA-TE) for coordination of this effort throughout the community and comment adjudication, Dr. Gene Stark (JPM P) for guidance from the technical program management perspective, Charlie Walker (WDTC) for assistance with comment adjudication of the test plan, Robin Gent and Julia Leadore (SURVICE Engineering Company; Belcamp, MD) for administrative support, Michael Sheely (ECBC) for analysis of all the samples, and Catherine Stern (ECBC) for quality oversight of this test program. The data from this verification testing has been recorded in ECBC notebook number 14-0001, entitled *LVAP V&V*.

Blank

DEPARTMENT OF THE ARMY
OFFICE OF THE DEPUTY UNDER SECRETARY OF THE ARMY
102 ARMY PENTAGON
WASHINGTON, DC 20310-0102

DUSA-TE

APR 30 2015

MEMORANDUM FOR DISTRIBUTION

SUBJECT: Approval of the Individual Protection (IP) Capability Area Process Team (CAPAT) Low-Volatility Agent Permeation (LVAP) Verification and Validation (V&V) Report

1. Reference: Memorandum, DUSA-TE and 19 July 10, subject: Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan.

2. The Test and Evaluation Capabilities Integrated Process Team (TECMIPT) reviewed the enclosed report and all Individual Protection Capability Area Process Action Team (CAPAT) members concurred with the data it contains. Upon the recommendation of the TECMIPT Chair, and in accordance with the reference, I approve the final V&V report and the use of LVAP in future individual protection assessments across the CBDP Enterprise.

3. My point of contact for this action is Ms. Deborah Shuping, (703) 545-1119, deborah.f.shuping.civ@mail.mil.

Encl

JAMES C. COOKE
CBRN Defense T&E Executive

DISTRIBUTION:
DASD(CBD)
DASD(DT&E)
DOT&E, Deputy for Land and Expeditionary Warfare
Army G3/5/7
Army G8 (DAPR-FDZ-I)
AF-TE
CNO, N091
Commander, ATEC

DUSA-TE
SUBJECT: Approval of the Individual Protection (IP) Capability Area Process Team (CAPAT) Low-Volatility Agent Permeation (LVAP) Verification and Validation (V&V) Report

DISTRIBUTION (CONT)
Commander, AFOTEC
Commander, OPTEVFOR
JPEO-CBD
JRO- CBRND
JSTO-CBD
Director, NSRDEC
Director, USANCA
DTRA/JSTO-CB
Director, ARL/SLAD
Technical Director, ECBC
Director, MCOTEA
Director, CBDP PAIO
Commander, NSWC-DD

DEPARTMENT OF THE ARMY
OFFICE OF THE DEPUTY UNDER SECRETARY OF THE ARMY
102 ARMY PENTAGON
WASHINGTON, DC 20310-0102

DUSA-TE

MEMORANDUM FOR Chemical, Biological, Radiological and Nuclear Defense Test and Evaluation Executive, Office of the Deputy Under Secretary of the Army (DUSA-TE), Taylor Building, Suite 8070, 2530 Crystal Drive, Arlington, VA 22202

SUBJECT: Low-Volatility Agent Permeation (LVAP) Verification and Validation (V&V) Report

1. The Individual Protection (IP) Capability Area Process Action Team (CAPAT), along with interagency stakeholders, completed the V&V report in accordance with DUSA-TE instructions to the TECMIPT, the Standards Development Plan, and the TECMIPT Standard Operating Procedure. All signatory members of the CAPAT have provided their concurrence to the attached V&V Report.

2. Based on the concurrence of the CAPAT, I recommend the CBRN Defense T&E Executive approve this V&V report as a Department of Defense Test and Evaluation Standard.

Encl

SEAN P. O'BRIEN
TECMIPT Chair

Individual Protection Capability Area Process Action Team (IP CAPAT)
Low-Volatility Agent Permeation (LVAP) Verification and Validation (V&V) Report
Concurrence Sheet

The IP CAPAT recommends approval of the LVAP V&V report. If a representative non-concurs, a dissenting position paper will be attached.

Organization	Signature	Date
Deputy Under Secretary of the Army Test and Evaluation (DUSA-TE)	Sean P. O'Brien	3/4/2015
Joint Program Executive Office of Chemical Biological Defense (JPEO-CBD) Test & Evaluation	Mark F. Thomas	3/16/2015
Joint Requirements Office for Chemical, Biological, Radiological and Nuclear Defense (JRO-CBRND)	For Lt Col Laurie K. Richter, USAF	4/6/2015
Joint Science and Technology Office (JSTO)	ROBERTS.MICHAEL.A.1228803371 Digitally signed by ROBERTS.MICHAEL.A.1228803371 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=DTRA, cn=ROBERTS.MICHAEL.A.1228803371 Date: 2015.03.04 21:30:04 -05'00' Michael A. Roberts	
US Army Evaluation Command (AEC)	FISHER.TIMOTHY.WILLIAM.1166077830 Digitally signed by FISHER.TIMOTHY.WILLIAM.1166077830 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=FISHER.TIMOTHY.WILLIAM.1166077830 Date: 2015.03.02 18:12:45 -05'00' LTC Timothy W. Fisher	
Operational Test and Evaluation Force (OPTEVFOR)	Jeffrey L. Bobrow	9 Mar 15
Air Force Operational Test and Evaluation Center (AFOTEC)	Grant D. Schaber, Civ, DAF Acting Director of Operations	20 Feb 15
Marine Corps Operational Test & Evaluation Activity (MCOTEA)	LtCol Kevin P. Reilly	17 MAR 2015
IP CAPAT Co-Chair	Robert G. Van Alstine	19 Mar 2015
IP CAPAT Co-Chair	Ryan B. Adams	26 Feb 2015

EXECUTIVE SUMMARY

This report provides details for the verification and validation (V&V) of a low-volatility agent permeation (LVAP) test methodology. Upon acceptance of this V&V report, this methodology will be transitioned to the Test and Evaluation (T&E) community for use in current and future acquisition programs. LVAP test methods have been shown to be more accurate for measuring the permeation of low-volatility contaminants such as *O*-ethyl *S*-[2-ethyl] methylphosphonothioate (VX). Traditional methods using a liquid challenge and a vapor sample collection are problematic when applied to low-volatility compounds. The method results detailed in this report were derived from multiple years of research at the U.S. Army Edgewood Chemical and Biological Center (ECBC; Aberdeen Proving Ground [APG], MD) with support from the Joint Science and Technology Office (JSTO; Ft. Belvoir, VA), U.S. Army Natick Soldier Research, Development, and Engineering Center (NSRDEC; Natick, MA), and the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD; APG, MD).

LVAP was developed several years ago at ECBC, in support of JSTO and Joint Project Manager for Nuclear, Biological, and Chemical Contamination Avoidance (JPM NBC CA) programs, to promote the safety of workers handling low-volatility contaminants. This method builds on the requirements of the TOP 8-2-501A expulsion test,[1] adding the capabilities of quantification and temperature control. A contact weight on top of the contaminated swatch ensures that contact occurs between the swatch and sorbent pad layers. This contact is critical for accurate measurement of agent permeation through the swatch.

After its initial development, the LVAP method was used for Science and Technology (S&T) V&V studies, in support of Joint Project Manager for Protection (JPM P) and JSTO programs. These recent S&T V&V efforts have shown acceptable statistical variability between laboratories for air-permeable materials that met test plan criteria. However, the test method had been modified since the original S&T development, and it was found unsuitable for air-impermeable materials because wicking of the liquid contaminant over the edge of the swatch caused false-positive results.[2]

Stakeholders from the Chemical and Biological Defense program community, including representatives from ECBC, Battelle, JPM P, JPEO-CBD, and Deputy Under Secretary of the Army for Test and Evaluation (DUSA-TE), worked together to address this issue. The solution identified for the wicking issue involves using a smaller contact region and leaving a buffer zone between the contaminant and the edge of the swatch. The effort detailed in this report establishes the V&V for the most recent configuration, which allows the method to be used for air-permeable and air-impermeable materials.

This V&V effort leverages the lessons learned from previous efforts and documents a single method for use by the T&E community. The data package for this V&V report is compliant with the requirements listed in the DUSA-TE memo, *Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan*, dated 2010.[3]

The test method performance was characterized through calculation of the intermediate-precision standard deviation (IPSD) via a single-laboratory study at ECBC, as detailed in Section 6.4.[4] The International Organization for Standardization (ISO) method, 5725-3 (1994), was used to calculate the standard deviation of the method when executed by a single laboratory, where certain parameters were held constant and others were allowed to vary. Parameters held constant were the laboratory, operators,

---

[1] *Test Operations Procedure (TOP) 8-2-501A, Permeation and Penetration of Air-Permeable, Semipermeable, and Impermeable Materials with Chemical Agents or Simulants*; TOP-8-2-501A; West Desert Test Center: Dugway Proving Ground, UT, 2013; UNCLASSIFIED Procedure.

[2] Stickel, G.; Andrews, A.; MacIver, B.; Steinbach, C. *Verification and Validation Test Report for Low Volatility Agent Permeation Test Method*; Customer Report to JPM P and NSRDEC, 2012.

[3] *Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan*; Deputy Under Secretary of the Army for Test and Evaluation: Arlington, VA, 2010.

[4] *Accuracy (Trueness and Precision) of Measurement Method and Results—Part 3: Intermediate Measures of the Precision of a Standard Measurement Method*; *5725-3:1994(E)*; International Organization for Standardization: Geneva, Switzerland, 1994.

and test equipment. Parameters allowed to vary were the test day and the analytical calibration, given that a new calibration curve was generated for each test day. The IPSD was calculated for both the air-permeable (24 h only) and air-impermeable (24 and 48 h) materials. The IPSD provided the expected variability that the method would have within a single laboratory on a day-to-day basis, calculated with well-known swatch samples. The calculated IPSD values are presented in the table. These values include all relevant data for the material.

Table. LVAP-Calculated IPSD for Single-Laboratory Testing: All Test Data

Material	Contact Time (h)	S_r: Single-Laboratory Within-Test-Day Standard Deviation (Repeatability) (%)	S_L: Between-Test-Day Standard Deviation (%)	IPSD (%)
Polytetrafluoroethylene control for dosing tools	n/a	1.2	5.8	5.9
APC01	24	83.6*	22.9*	86.8*
Latex	24	5.2	6.3	8.2
	48	4.6	2.1	5.0

* APC01 had a single data point that was approximately 6 times higher than the mean, but there was no attributable cause for removal. Removing this outlier dramatically changed the results to 13.8, 13.2, and 19.1%. n/a, not applicable.

The single-laboratory S_r designation was used to clarify that this repeatability estimate was not based on a multi-laboratory study. In the APC01 tests, a single result that was approximately 6 times greater than the mean dramatically skewed the calculations. Additional information is provided in Section 4.3.

Additional calculations, presented in Section 7, suggest that the variability is dependent on the material type and the permeation performance. High-performance materials lead to low-concentration samples, which have inherently greater variability upon analysis.

The test plan for the V&V was established with input from ECBC, West Desert Test Center (Dugway Proving Ground, UT), JPM P, DUSA-TE, and the Individual Protection Capability Area Process Action Team (IP CAPAT) personnel. The test date schedule is provided in Section 2.11. The V&V process was accelerated to enable the Contaminated Human Remains Pouch (CHRP) program personnel to leverage the LVAP test method as part of the program.

To enable CHRP program personnel to use the LVAP as a validated test method to address programmatic testing requirements for VX, the V&V needed to be conducted before all signatures had been received from all stakeholders. In an effort to mitigate the risk of this data not being accepted by the T&E community, the test plan was sent to the IP CAPAT and Operational Test Agencies (OTAs) for review in March 2014. All captured comments were adjudicated. Approval to move forward with the test plan execution was obtained from DUSA-TE, JPM P, Marine Corps Operational Test and Evaluation Activity (MCOTEA), and Operational Test and Evaluation Force (OPTEVFOR). The results of the verification testing were presented to the Test and Evaluation Capabilities and Methodologies Integrated Process Team (TECMIPT) in April 2014, and a Technical Readiness Review (TRR) was conducted in June 2014. Written approval to conduct validation testing following the TRR was received from JPM P and the U.S. Army Test and Evaluation Command (ATEC).

CONTENTS

FIGURES

TABLES

LOW-VOLATILITY AGENT PERMEATION (LVAP) VERIFICATION AND VALIDATION REPORT

1. INTRODUCTION

1.1 Objective

The objective of this effort was to establish low-volatility agent permeation (LVAP) as a verified and validated test methodology, using a data package compliant with the requirements listed in the Deputy Under Secretary of the Army for Test and Evaluation (DUSA-TE) memo, *Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan*, dated 2010.[1] Upon approval by the Chemical and Biological Defense community, LVAP will be transitioned to the Test and Evaluation (T&E) community for use in current and future acquisition programs.

1.2 Intended Use

The report for this verification and validation (V&V) will document the procedures, parameters, and standard deviation associated with *O*-ethyl *S*-[2-ethyl] methylphosphonothioate (VX) permeation through air-permeable and air-impermeable materials at a 10 g/m^2 challenge for a 24 h contact scenario at 32.2 °C, and for air-impermeable materials at a 10 g/m^2 challenge for a 48 h contact scenario at 32.2 °C. Ultimately, Department of Defense (DoD) LVAP test capabilities will exist at the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) and the West Desert Test Center (WDTC; Dugway Proving Ground, UT).

1.3 Background

Research efforts examining the permeation behavior of VX have demonstrated that liquid contamination–vapor detection methods do not accurately characterize the quantity of contaminant that has permeated the swatch. A contact method was established for low-volatility contaminants.[2] Recent Science and Technology (S&T) V&V efforts have shown acceptable statistical variability between laboratories for air-permeable materials.[3] However, the test method had been modified from the original test methodology, and it was found unsuitable for air-impermeable materials. Changes in polytetrafluoroethylene (PTFE; e.g., Teflon) disk size and agent droplet pattern caused liquid contaminant to wick over the edge of the swatch, producing a false-positive result. Recent efforts to resolve the wicking issue include using a smaller contact region and creating a buffer zone between the contaminant and the edge of the swatch. This plan establishes the V&V for the most recent configuration and is acceptable for use with both air-permeable and air-impermeable materials.

1.4 Capabilities, Assumptions, Limitations, Risks, and Impacts

1.4.1 Capabilities

As a capability, laboratories and operators who use the LVAP method will obtain accurate measurements of the total mass of low-volatility agent that has permeated air-permeable and air-impermeable test swatches. These more accurate measurements will provide benefit to protection programs that rely on T&E data to make programmatic and milestone decisions and will ultimately benefit the Warfighter.

1.4.2 Assumptions

It was assumed that the laboratory operators conducting these procedures were skilled at handling surety materials, had been trained in performing the steps detailed in this document, and were capable of analyzing low-level samples. These same assumptions would apply to other laboratories that plan to use this test plan to become validated in this LVAP test method.

It was assumed that the moisture-uptake measurements obtained during the preconditioning verification were representative of the preconditioning for all air-permeable swatches of this material. For the purposes of this test process, the measured level of moisture was assumed to be the same for the validation and future testing for this material. Preconditioning conditions were logged to demonstrate the temperature and humidity conditions during the V&V tests.

It was assumed that a system that met the temperature verification requirements for 24 h would also be able to meet them for 48 h. Temperature verification testing for 48 h was not performed. Details of testing conducted during 48 h validation test periods were recorded to verify this assumption.

1.4.3 Limitations

The LVAP method is solely a materials-level test that is applicable to testing swatches of air-permeable or air-impermeable materials under static conditions. The test plan did not account for testing of materials under stress load conditions.

It is a test limitation that this method may not be appropriate for contaminant-repellent materials because these materials do not absorb contaminants.

LVAP measures the cumulative permeation during the test period as a single data point; as such it is not a near-real-time method.

Low levels of VX vapor were previously detected over the course of a 24 h test. This background level of contaminant collected on the sorbent pad may have affected the practical limit of quantification. The degree of impact would depend on the target threshold and objective levels for a given program. Methods were documented as part of the verification process to establish the efficacy and effectiveness of the gasket seal and the impact on permeation testing.

It should be noted that a single lot of divinyl benzene (DVB) sorption pads was not available for all V&V testing. Various lots of DVB pads were used throughout the testing, and the lot numbers were noted on the test sheets as part of the documentation process.

1.5 Safety Considerations

Personnel from the ECBC offices of Safety and Health, and Environmental Quality completed the required preoperational surveys and hazard analyses in support of these test processes. Before testing began, standard operating procedures were developed to cover all aspects of testing, including general and unique operations, surety and toxic material handling, decontamination, disposal, evacuation, and emergency response. All technical and support personnel received extensive training in the requisite procedures to ensure the safe handling of hazardous and toxic substances. Periodic safety inspections were performed throughout the testing. The ECBC safety officers ensured that all approved safety procedures were properly implemented and enforced.

1.6 Tolerances

The targeted values for each parameter and the acceptable tolerances are shown in Table 1. References for the targets and tolerances are also provided. The target for the stainless steel weight was obtained from TOP 8-2-501,[4] but no tolerance level was provided within that document. In this case, the tolerance was derived from best manufacturing practices. The 30% tolerance level for the sorbent pad efficiency was taken from a U.S. Environmental Protection Agency (EPA) method, where the same pad type was used, Empore type SDB-XC extraction disk (3M; St. Paul, MN). This model of sorption pad was identical to the one used in the testing.

Table 1. Target Values and Tolerances

Component	Measurement	Target	Tolerance	Reference
Weights	Mass	453.6 g (1.00 lb)	±5 g (±0.01 lb)	TOP 8-2-501[4]
	Dimensions	28.651 mm diameter 3.277 mm nub length	±0.254 mm (±0.010 in.)	
Preconditioning chamber	Temperature	32.2 °C (90 °F)	±1.1 °C (±2 °F) for 95% of total readings	
	Relative humidity	80%	±5% for 95% of total readings	
	Absolute humidity	28.3 g/m^3	±3.4 g/m^3 for 95% of total readings	
Test chamber	Temperature	32.2 °C (90 °F)	±1.1 °C (±2 °F) for 95% of total readings	
Uptake efficiency	Average recovery % compared to target	100%	±30% target	EPA SW-846[5]
Extraction efficiency		100%	±30% target	
Operator proficiency		100%	±15% target	EPA Method 8000B[6]
Analytical	Calibration curve	100%	±20% target	
	Continuing calibration verification	100%	±15% first sample, within 10% of initial for subsequent samples	
Purity	Agent purity	>90%	>90%	n/a

n/a, not applicable.

2. SYSTEM DESCRIPTIONS

2.1 Test Materials

The test materials for this effort included the following:

- Butyl rubber from 7 mil butyl gloves, manufactured in accordance with MIL-DTL-43976D.[7] Because this material was used for control swatches and not for testing permeation performance, swatches were taken only from the palm and back regions of the gloves.
- Latex from 10 mil, medium-soft (40A durometer), natural latex rolled sheets (part no. 85995K14; McMaster-Carr; Elmhurst, IL). The thickness tolerance was ±0.002 in.

- Neoprene from 17 mil, 50 ± 5 durometer, black neoprene rolled sheets (part no. CASS-.017X36-35000; AAA-Acme Rubber Company; Tempe, AZ). The thickness tolerance was ±0.010 in.

Air-impermeable materials were cut using a 50 mm cutting die and press. The exact swatch diameter did not impact the LVAP test.

Air-permeable controls were from material APC01, which was supplied by the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD; Aberdeen Proving Ground, MD). As received, this material was prewashed and precut into swatches. Verification testing with material APC01 was limited to preconditioning steps; permeation testing was not conducted with this material during verification testing. Permeation experiments with APC01 were conducted during validation testing.

Impermeable material swatch thicknesses were measured prior to verification testing. For validation testing, thickness measurements were limited to latex swatches.

2.2 Preconditioning Chamber

The preconditioning chamber consisted of a polycarbonate box with wire shelves to hold air-permeable swatches in preparation for testing. The box was placed in an environmental chamber, where conditioned temperature- and humidity-controlled air flowed through it. Prior to testing, the temperature and humidity parameters were established in accordance with test requirements to attain the proper moisture-content equilibrium in the swatches during the 24 h preconditioning phase. Calibrated temperature and humidity sensor systems recorded the conditions within the box during preconditioning. The performance of the preconditioning chamber was characterized as described in Section 3.1.

2.3 Test Chamber

The test chamber was an incubator that maintained the test temperature. A data logger and calibrated temperature probe were used to collect temperature information during testing. Humidity was not controlled within the test chamber, as each test cell was sealed, which created an isolated environment for each swatch. The incubator had been modified, with the addition of sliding shelves, to facilitate test cell placement and removal. Before permeation testing was started, the temperature of the areas inside the test chamber, where the test cells were placed, was characterized and mapped as detailed in Section 3.2.

2.4 Test Cells

Each test cell consisted of a PTFE-lined polycarbonate Petri dish, a sorbent pad, a swatch, a 28 mm PTFE disk, and a 453.6 g stainless steel weight contained within an inverted 240 mL glass jar. A schematic is shown in Figure 1. During permeation characterization of some samples, a gasket O-ring (Buna-N O-ring, part no. 224N70; Paramount Packing and Rubber; Baltimore, MD) was placed on the contaminated swatch before the weight was applied. The gasket had a nominal outer diameter of 2.0 in. and a nominal inner diameter of 1.75 in. The O-ring served as a gasket, sealing against the stainless steel weight to prevent vapor cross-contamination. The O-ring was used in all subsequent permeation samples for validation testing. Additional information is provided in Section 3.9.

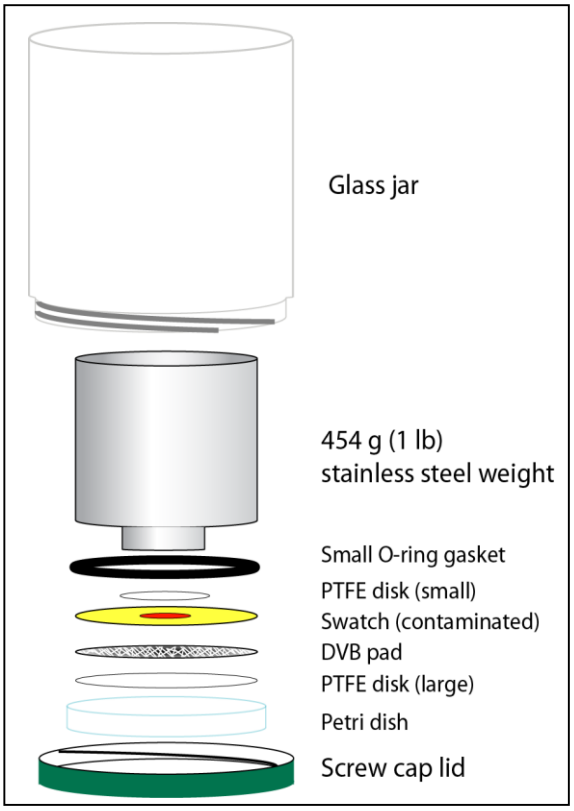


Figure 1. The new contact test fixture (patent pending).

2.5 Weights

Using weights ensured that contact occurred between the swatch and the DVB sorbent pad. The weights were made of stainless steel and designed to apply 1 psi to the swatch. Direct pressure was needed to ensure good contact.[2] Additional requirements are listed in Section 4.5.

2.6 Solid Sorbent Pads

The DVB pads (Empore type SDB-XC, with a 47 mm diameter) were the matrix for collecting the permeated agent. At the conclusion of each test, the pad was extracted, and an aliquot was analyzed to measure the total mass of contaminant. The lot number of the DVB pad used for each test was noted on the run sheets, which are provided in Appendix A. For most of the testing, the DVB disks were used as received, without activation procedures. Some pads were activation processed during an efficiency scoping test, Test I, to document the effect of the activation process. The uptake and extraction efficiencies were documented for three contamination levels. The characterization steps are detailed in Section 3.7.

2.7 Agent

VX was the contaminant used for this test. The minimum purity requirement was 90%. Lot VX-U-1223-CTF-N was used, which had a purity >90%; however, this material was not a Chemical Agent Standard Analytical Reference Material (CASARM). Detailed purity information is provided in Section 5.2. The certificate of analysis (CoA) is provided in Appendix B.

2.8 Spiking Tool

Contaminant was applied using a 50 µL gas-tight syringe with blunt-tip needle. A 1 µL droplet volume was generated by using a 1/50 repeating dispenser tool. A six-drop pattern was contained within a 6 cm^2 dosing region in the center of the swatch, as shown in Figure 2. This pattern produced a contamination density of 10 g/m^2, was shown to be effective at preventing liquid wicking, and had the lowest background vapor levels recorded during recent S&T evaluation tests. This pattern, including the 1 µL drop volume, was similar to that used by the Aerosol, Vapor, Liquid Assessment Group (AVLAG). However, AVLAG used 10 droplets within a 10 cm^2 contamination area, whereas LVAP used 6 droplets within a 6 cm^2 contamination area. The contact region for the weight was the same as the dosing region boundary and contamination area.

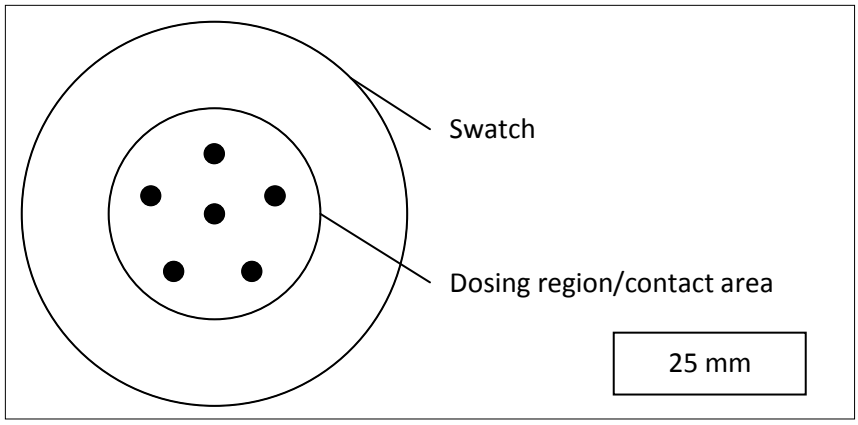


Figure 2. Dosing region and drop pattern for contaminating swatches.

2.9 Solvents

Acetone was used for standard preparation, dilute contaminant application during efficiency evaluations, and VX extraction from the DVB sorbent pads. In initial work with acetonitrile and methanol, extraction efficiencies were less optimal than those obtained using acetone. All solvents were high-performance liquid chromatography (HPLC) grade or better.

2.10 Analysis Equipment

The analytical instrumentation for sample analysis was liquid chromatography-tandem mass spectrometry (LC-MSMS), which has been shown to be more sensitive and more stable than gas chromatography methods for VX analysis. Additional requirements and analytical limits of quantification are provided in Section 4.3.

2.11 Test Schedule

Each V&V test that required the use of agent was assigned a letter code to facilitate sample processing and data archiving. The test matrix is provided in Table 2.

Table 2. Verification and Validation Test Matrix with Letter Codes

Test Type	Test ID	Description	Date Conducted
Verification	A	24 h efficiency verification 240 mL jar (acetonitrile)	25-Feb-14
	B	24 h efficiency verification 60 mL jar (acetonitrile)	25-Feb-14
	C	Operator proficiency	10-Mar-14
	D	Characterization verification	26-Mar-14
	I	Extraction efficiency scoping (acetone and methanol)	11-Mar-14
	J	24 h efficiency verification 60 mL jar (acetone)	13-Mar-14
	K	Characterization verification Repeat	8-Apr-14
	L	48 h efficiency verification 60 mL jar (acetone)	15-Apr-14
Validation	E	24 h Validation Test 1 Latex and APC01	9-Jul-14
	F	24 h Validation Test 2 Latex and APC01	22-Jul-14
	H	48 h Validation Test 1 Latex	29-Jul-14
	M	48 h Validation Test 2 Latex	18-Aug-14
	N	24 h Validation Test 3 Latex and APC01	16-Sept-14

3. VERIFICATION TESTING

3.1 Swatch Preconditioning

The steps for verifying the performance of the individual components and the system as a whole are described in this section. For verification tests that required the use of agent, a coversheet was included on the run sheet for that particular test to document pertinent test information.

3.1.1 Swatch Preconditioning Chamber

Swatch preconditioning is the process of adjusting the moisture level within an air-permeable swatch. Active carbon permeation performance is highly affected by moisture content. Therefore, all air-permeable swatches were preconditioned to ensure that the swatches were at the same conditions and thereby supported accurate comparisons. This verification test documented that the temperature and relative humidity (RH) were controlled within acceptable limits.

The preconditioning chamber was a box built from 0.25 in. thick polycarbonate sheets. The total volume of the chamber was approximately 25 L. The chamber had two stainless steel wire shelves, each of which was equipped with 20 stainless steel spring clips. The shelves were configured to allow for airflow, exposing all portions of the swatch to the preconditioned air. The clips holding each swatch were individually numbered, which allowed for each swatch to be tracked through the preconditioning process.

A rubber gasket was placed around the top rim of the chamber to create a seal when the lid was attached. Four draw-clasps were attached to seal the top lid to the base unit.

The preconditioning chamber is shown in Figure 3. Here, a single shelf and randomly placed swatches were included to illustrate the layout of the chamber.

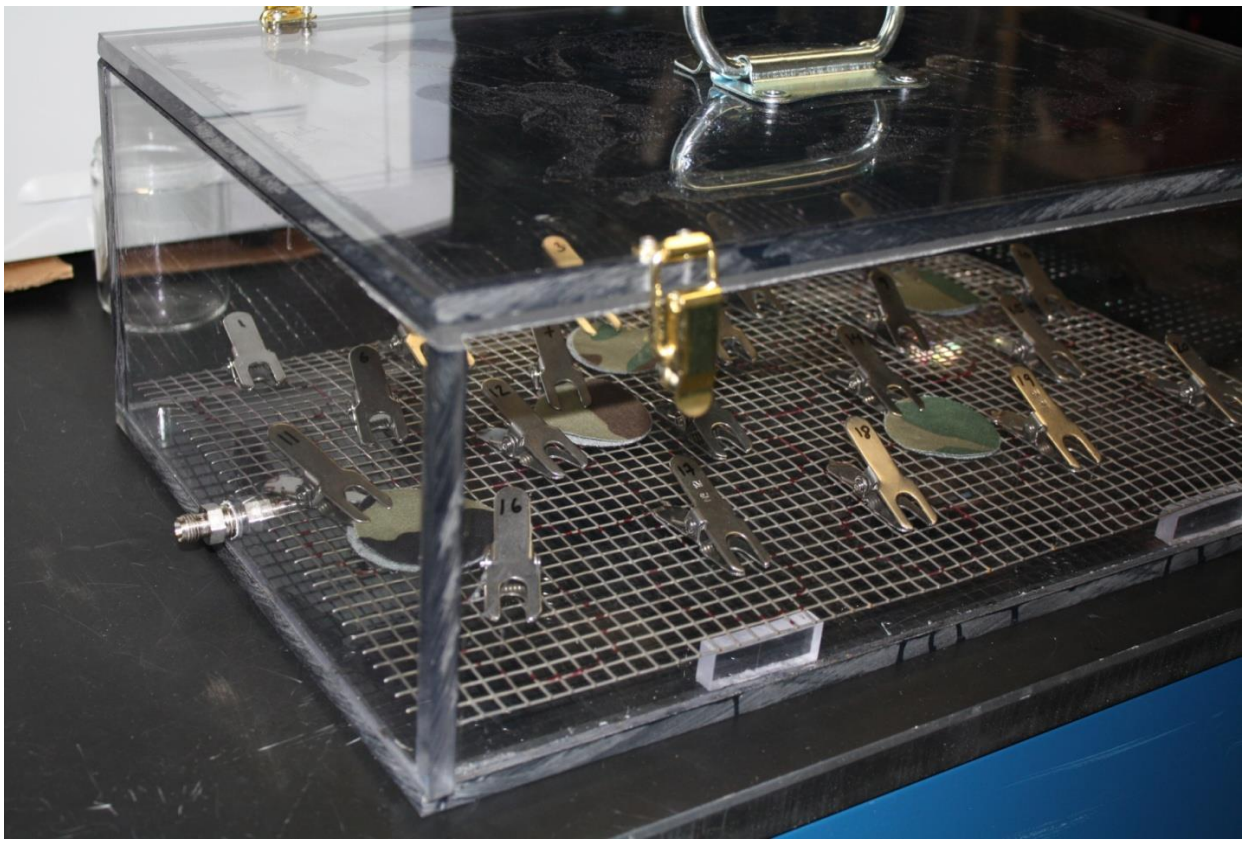


Figure 3. The polycarbonate preconditioning chamber, with one of the wire racks in place.

To precondition the swatches, the polycarbonate box was placed into an environmental chamber. Conditioned air (32 °C and 80% RH) was directed into the preconditioning chamber through Swagelok fittings (Swagelok Company; Solon, OH) at a rate of approximately 10 standard liters per minute (sLpm). Inlet and outlet air were monitored using calibrated National Institute of Standards and Technology (NIST)-traceable humidity and temperature data loggers. The conditions inside the preconditioning chamber were monitored at two locations using calibrated NIST-traceable measurement devices connected to data loggers. Details about the calibrated instruments used to characterize the preconditioning chamber are provided in Section 5.6.

3.1.2 Swatch Preconditioning Chamber Requirements

The target environmental set point for the swatch preconditioning chamber was 32.2 °C (90 °F) and 28.3 g/m^3 water absolute humidity (80% RH). The swatch preconditioning chamber operation was characterized to document control of the temperature and humidity within acceptable limits for a 24 h period, and the conditions were logged at least once every 2 min. Temperature and humidity were measured with calibrated sensors. The resolution was at least 0.1 °C for temperature and 1% for RH.

The minimum acceptance requirements for the preconditioning chamber included maintenance of the set temperature to within 1.1 °C of the temperature target and the set humidity to within 5% of the RH target for greater than 95% of the total readings.

The reporting requirements for the preconditioning chamber verification included two histogram plots and two time series plots, one each for temperature and humidity. The two histogram plots were required to show the relative percentage count versus temperature and the relative percentage count versus RH. The time series plots were required to be scatter plots of temperature or humidity versus elapsed time.

The summary temperature and humidity results are provided in Table 3. The temperature histogram is presented as Figure 4, and the temperature time profile plots are shown as Figure 5. The absolute humidity histogram is presented as Figure 6, and the absolute humidity profile over time is presented as Figure 7. The RH histogram is presented as Figure 8.

The device that measured and logged the outlet conditions stopped working 16 h into the trial. This malfunction did not impact testing, as the conditions within the preconditioning chamber remained constant and within required specifications as measured by other logging devices co-located with the swatches.

Table 3 and Figures 4–8 fulfill the reporting requirements for the preconditioning verification.

Table 3. Summary Temperature and Humidity Results for the Preconditioning Chamber Verification

Location	Temperature			RH			Absolute Humidity		
	Average (°C)	StDev (°C)	RSD (%)	Average (%)	StDev (%)	RSD (%)	Average (g/m^3)	StDev (g/m^3)	RSD (%)
Inlet	32.7	0.05	0.15	80.58	0.24	0.3	28.26	0.07	0.23
Outlet	32.2	0.04	0.12	83.32	0.17	0.21	29.00	0.02	0.07
Back upper right	32.67	0.02	0.06	83.48	0.27	0.33	29.27	0.09	0.31
Front lower left	32.74	0.02	0.06	80.9	0.27	0.33	28.45	0.09	0.32

StDev, standard deviation.
RSD, relative standard deviation.

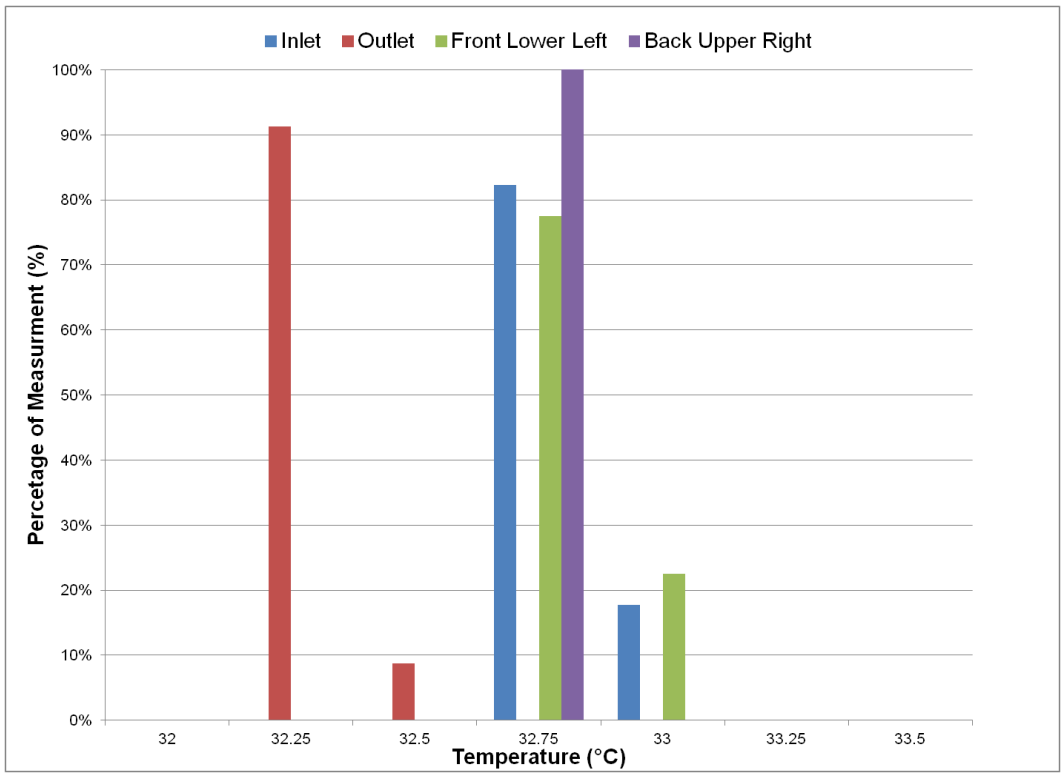


Figure 4. Temperature histogram for the preconditioning chamber verification.

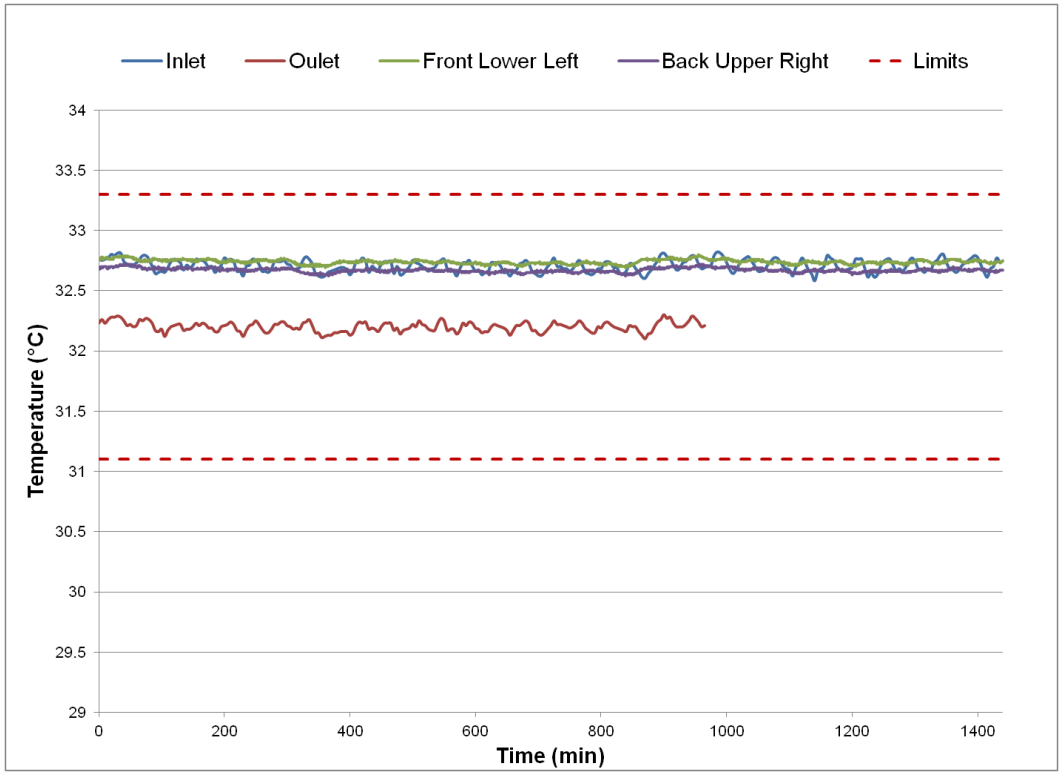


Figure 5. Temperature–time profile plot for the preconditioning verification.

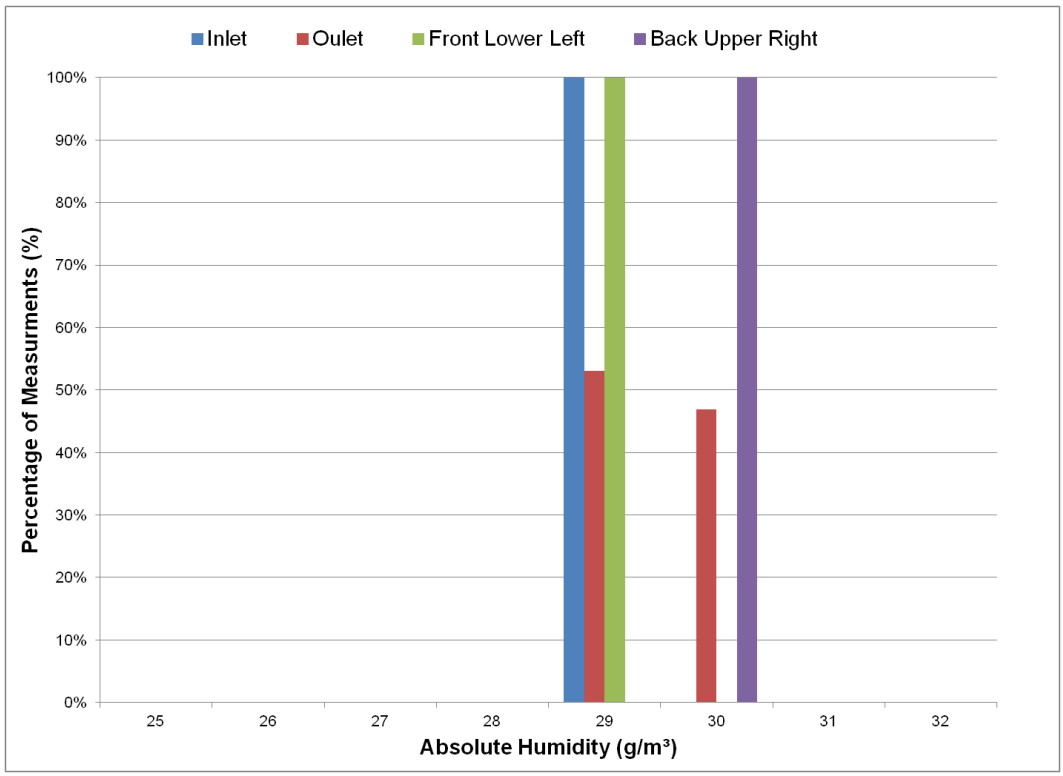


Figure 6. Absolute humidity histogram for the preconditioning chamber verification.

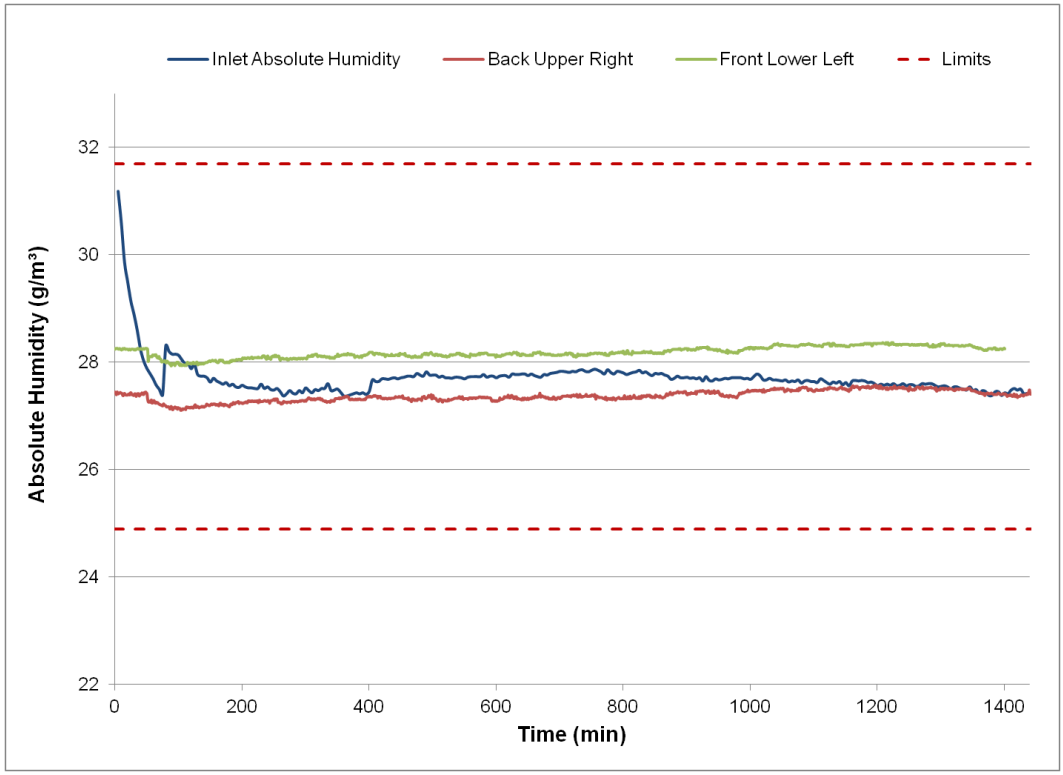


Figure 7. Absolute humidity–time profile plot for the preconditioning chamber verification.

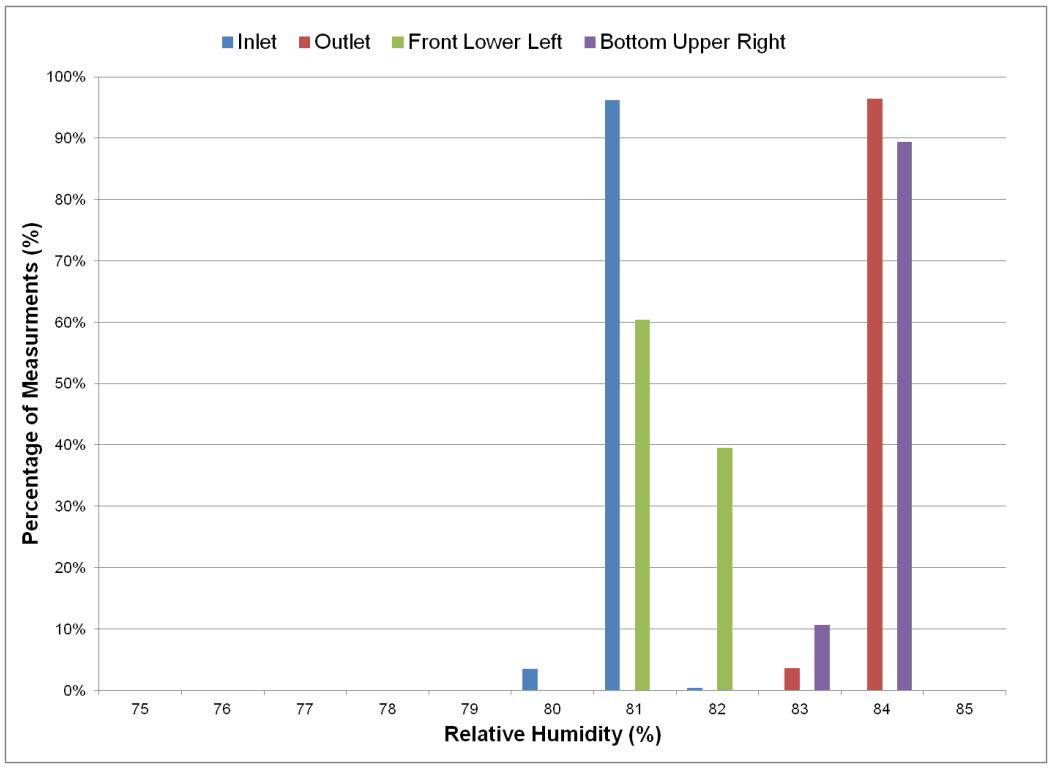


Figure 8. RH histogram for the preconditioning chamber verification.

3.2 Preconditioning Test on Swatches

The process of preconditioning swatches was only required for the air-permeable materials. The air-impermeable materials used in this testing (i.e., butyl, latex, and neoprene) were not affected by moisture levels, so moisture control was not required. Therefore, the preconditioning process for air-impermeable materials was not required (for temperature or humidity).

The process required that the air-permeable test materials be weighed prior to and after conditioning at the requisite temperature and humidity for 24 h.

To verify the preconditioning steps, a total of 20 air-permeable swatches were evaluated: 10 swatches were prepared with drying and preconditioning, and 10 swatches were prepared with preconditioning only. The test matrix is shown in Table 4.

Table 4. Swatch Preconditioning Verification Test Matrix

No. of Replicates	Dried	Conditioned at 32.2 °C and 80% RH	Weighed
10	Yes	Yes	Yes
10	No	Yes	Yes

A power calculation was performed to determine the minimum detectable difference between the dried and conditioned swatches versus the conditioned-only swatches for a given sample size.[8] Assuming a β of 0.2 and a standard deviation of 0.0091, 10 replicates of each swatch type were required to detect a difference of >0.02 g with 80% confidence if, in fact, such a difference did exist. The power-curve plot in Figure 9 shows the minimum mass difference that can be detected based on the number of replicate samples.

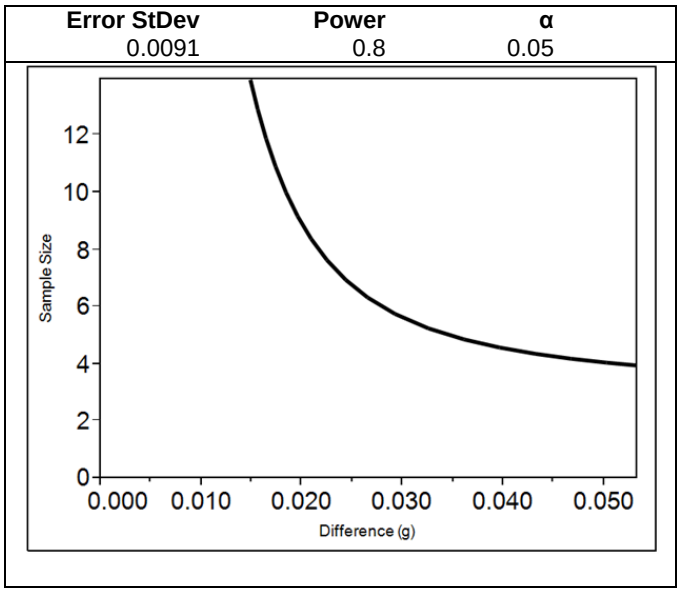


Figure 9. Power curve for mass water-uptake measurements.

A NIST-traceable calibrated analytical balance was used to obtain the masses of 20 swatches. A subset of 10 swatches was dried within the preconditioning chamber at 32.9 °C under a dry airstream, with <4% RH, for 24 h. After 24 h, the swatches were placed into a sealed container and transported to a NIST-traceable calibrated analytical balance to document the dry mass. Next, the swatches were returned to the preconditioning chamber along with the remaining ambient-conditioned (i.e., no pre-drying) swatches. The swatches were distributed within the chamber to remove placement bias, and the positions were documented. The conditions were set to 32.2 °C and 80% RH. The temperature and RH were documented with calibrated probes connected to data loggers. Swatches were conditioned for 24 h.

The summary of temperature and humidity results for the swatch drying process are presented in Table 5. The drying-stage temperature and absolute humidity profile plot is shown in Figure 10. It should be noted that the data logger stopped working after approximately 21 h of drying. The swatches were actually dried for 24 h, and environmental control was maintained during the entire time.

The summary results for the swatch conditioning are presented in Table 6. The RH histogram is presented as Figure 11. The temperature histogram is presented as Figure 12, and the temperature-time profile plots are shown as Figure 13. The absolute humidity histogram is presented as Figure 14, and the absolute humidity profile over time is presented as Figure 15. The inlet temperature was higher than the initial target range. However, the sensors inside the preconditioning chamber indicated that the swatches reached the required target conditions.

After 24 h conditioning, the swatches were removed and placed into a sealed transport container. The swatches were transported to a calibrated analytical balance to record the post-conditioning swatch mass. All efforts were made to minimize the swatch exposure to ambient humidity. The mass data is presented in Table 7.

The reporting requirements included several tables and plots. The masses of water uptake for the dried and conditioned swatches were tabulated in one table. The final masses for the dried and conditioned swatches were tabulated, along with the final masses for the conditioned-only swatches, along with the results for the statistical analysis.

Two histogram plots and two time series plots were also required; one set was for temperature and the other was for RH. A histogram plot was provided for the relative percentage count versus temperature, and another plot showed the relative percentage count versus RH. The time-series plots were scatter plots of temperature or humidity versus elapsed time.

The minimum requirements for acceptance of the preconditioning chamber were maintenance of the set temperature to within 1.1 °C of the temperature target and maintenance of the set humidity within 5% of the RH target.

Tables 4–7 and Figures 9–15 fulfill the reporting requirements for the preconditioning on swatch verification. The chamber met the specifications for the temperature and humidity control of the preconditioning chamber with swatches present.

Table 5. Summary Temperature and Humidity Results: Swatch Drying

Location	Temperature			RH			Absolute Humidity		
	Average (°C)	StDev (°C)	RSD (%)	Average (%)	StDev (%)	RSD (%)	Average (g/m^3)	StDev (g/m^3)	RSD (%)
Inlet	32.90	0.12	0.38	0.03	0.001	4.2	0.01	0.0006	6.2

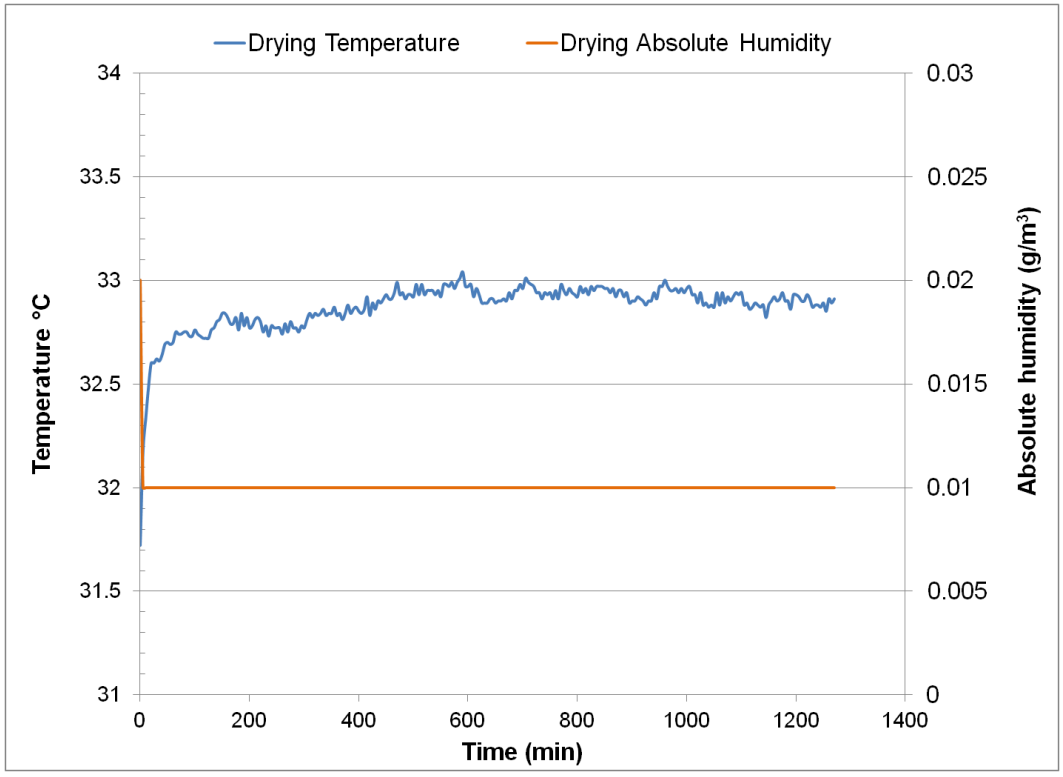


Figure 10. Temperature and humidity time profile plots: swatch drying.

Table 6. Summary Temperature and Humidity Results for the Preconditioning Chamber Verification

Location	Temperature			RH			Absolute Humidity		
	Average (°C)	StDev (°C)	RSD (%)	Average (%)	StDev (%)	RSD (%)	Average (g/m^3)	StDev (g/m^3)	RSD (%)
Inlet	32.90	0.08	0.23	78.13	0.92	1.18	27.69	0.26	0.95
Back upper right	32.69	0.09	0.27	78.05	0.42	0.53	27.37	0.10	0.36
Front lower left	32.53	0.08	0.26	81.07	0.39	0.48	28.18	0.10	0.36

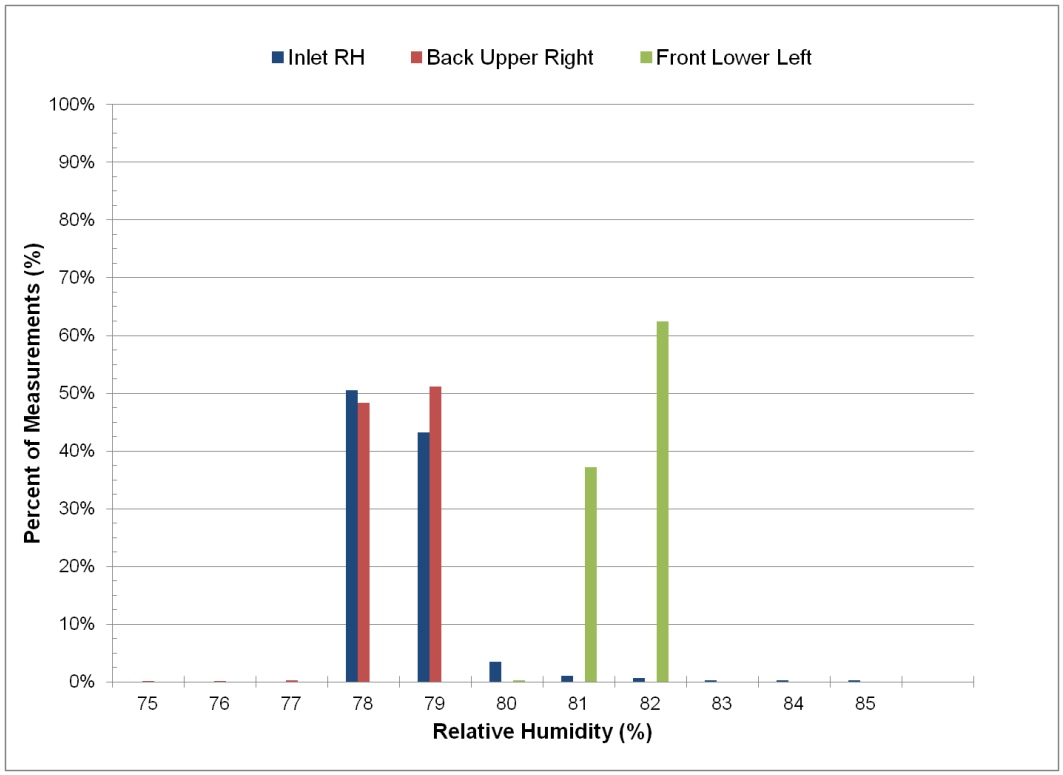


Figure 11. Swatch conditioning RH histogram.

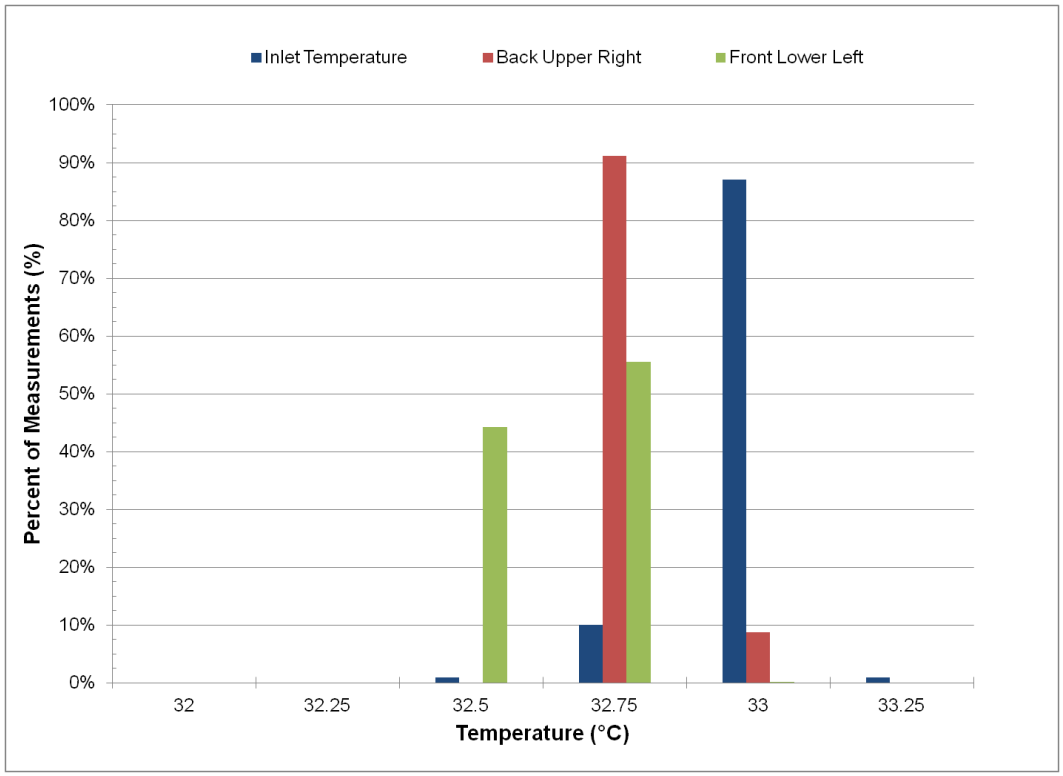


Figure 12. Swatch conditioning temperature histogram.

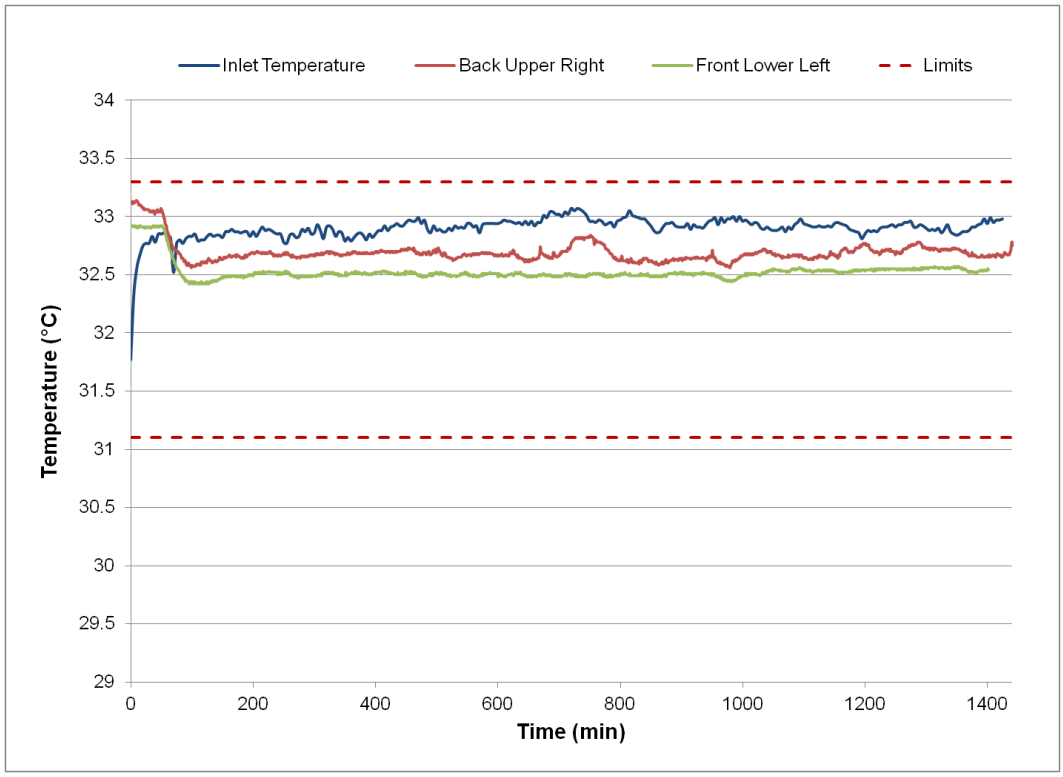


Figure 13. Swatch conditioning temperature–time profile plots.

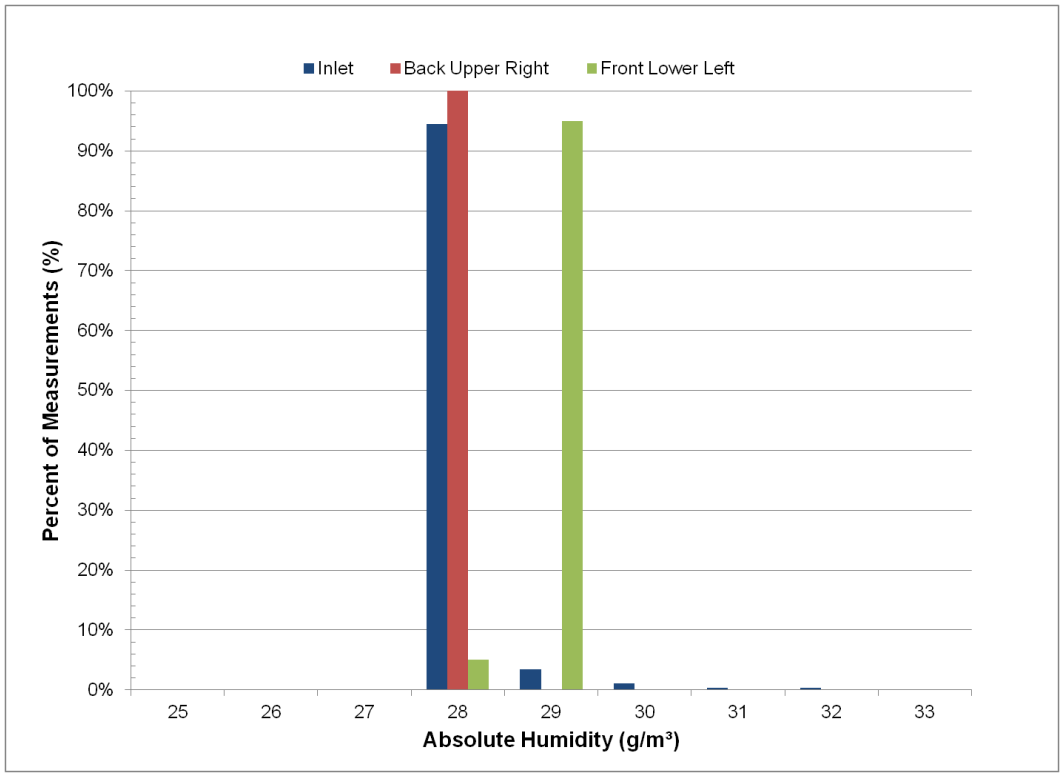


Figure 14. Swatch conditioning absolute humidity histogram.

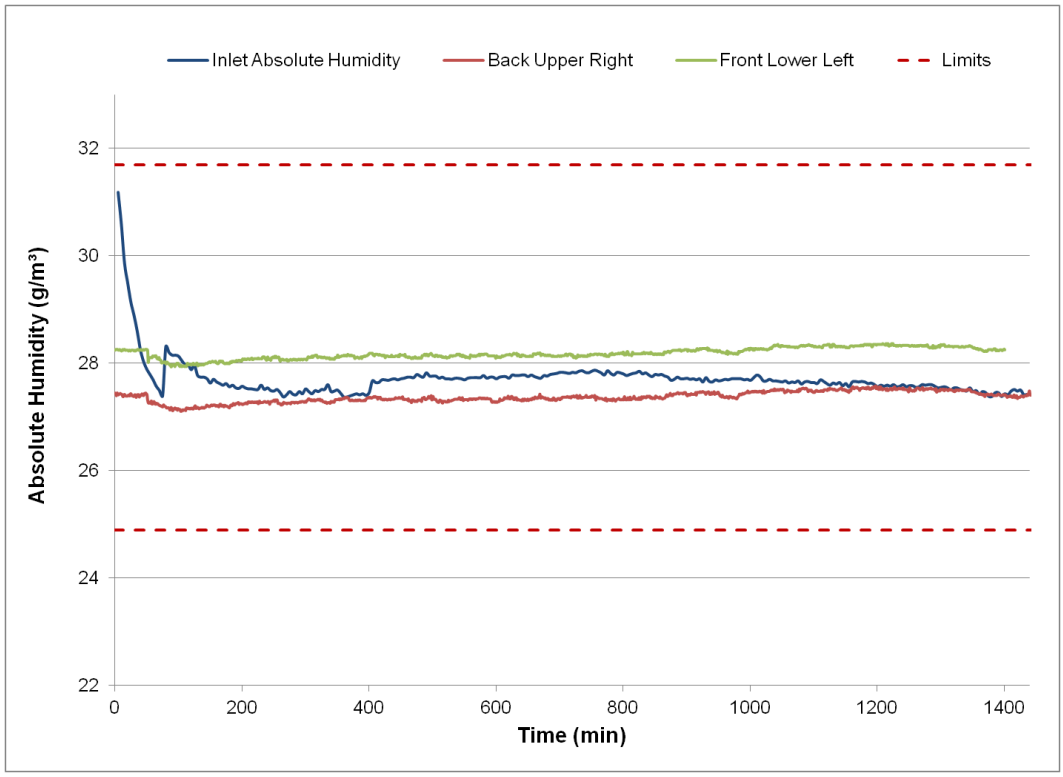


Figure 15. Swatch conditioning absolute humidity–time profile plots.

Table 7. Swatch Conditioning Water Mass Results

Set	Replicate	Position	Ambient Mass (g)	Dried Mass (g)	Conditioned Mass (g)	Water Uptake Mass (g)
Dried	1	1	1.25504	1.23307	1.40153	0.14649
	2	5	1.25276	1.23115	1.39554	0.14278
	3	14	1.26436	1.24802	1.41232	0.14796
	4	17	1.25965	1.24328	1.40845	0.14880
	5	20	1.26698	1.24982	1.41702	0.15004
	6	22	1.26144	1.24344	1.42051	0.15907
	7	25	1.25103	1.23292	1.41174	0.16071
	8	33	1.27913	1.25945	1.44455	0.16542
	9	36	1.26006	1.23976	1.42781	0.16775
	10	35	1.24663	1.22568	1.40984	0.16321
Nondried	1	21	1.25894	n/a	1.42033	0.16139
	2	24	1.23522		1.39798	0.16276
	3	28	1.23232		1.39772	0.16540
	4	32	1.25857		1.42776	0.16919
	5	39	1.24055		1.41714	0.17659
	6	2	1.25991		1.41207	0.15216
	7	4	1.22728		1.37722	0.14994
	8	8	1.23032		1.38215	0.15183
	9	12	1.22845		1.37597	0.14752
	10	19	1.25222		1.40984	0.15762

n/a, not applicable.

After the verification test was complete, the dried and nondried swatches were compared using an analysis of variance (ANOVA) single-factor analysis. The data failed to reject the null assumption that there was no statistical difference in the total water-uptake mass between the dried and nondried swatches. The *p* value for the water uptake was 0.317. Water-uptake data for each conditioning pathway is shown in Figure 16.

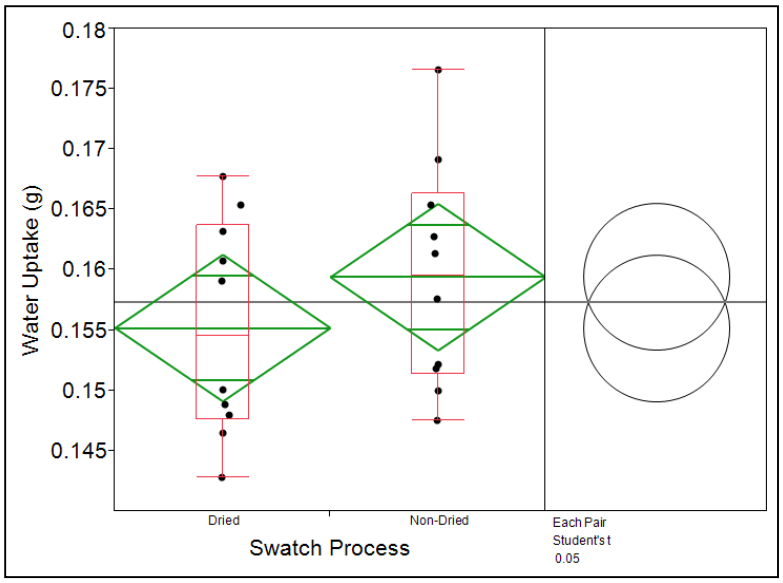


Figure 16. Graphical representation of water-uptake mass for dried versus nondried swatches.

3.3 Test Chamber Environmental Control

The test chamber was characterized across 10 locations using NIST-traceable calibrated temperature data loggers. The chamber was equipped with two shelves. Each shelf was characterized at five locations, four corners and the middle of each shelf, as shown in Figure 17. Each location was logged for 24 h at 1 min intervals with a resolution of 0.1 °C. The temperature data from the test chamber thermocouple was also logged. Figure 18 is a histogram that details the percentage of data points versus temperature. Figure 19 shows the temperature profile at each location over the 24 h (1440 min) test. Here, the dashed red lines indicate the temperature-control boundaries, and the orange bar represents the output from the test chamber internal thermocouple.

The reporting requirements for the incubator verification included two plots. The first was a scatter plot of temperature versus elapsed time for each location and the incubator log. The second was a histogram plot of the relative percentage count versus the temperature for each characterized location and the incubator log. The temperature range displayed was required to include all temperatures where a response was recorded that was more than 0.5% of the total relative percentage.

The minimum requirements for incubator acceptance consisted of two parts. First, there had to be less than 1.0 °C of temperature change between the average temperatures of each location, including the incubator log. Second, at each location and the incubator log, the set temperature had to be maintained to within 1.1 °C of the target for more than 95% of the total readings.

Figures 17–19 fulfill the reporting requirements for the test chamber environmental control verification. All measured data points were within the allowed tolerances.

Figure 17. Temperature-mapping probe locations within the test chamber.

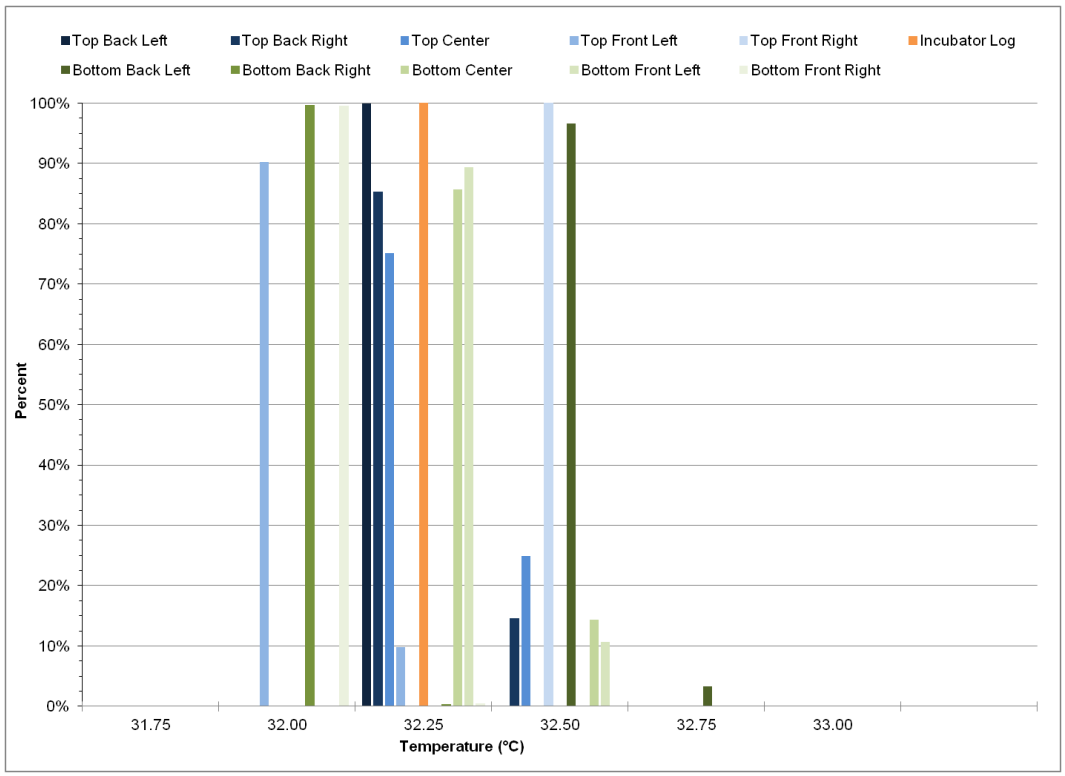


Figure 18. Results for test chamber temperature mapping.

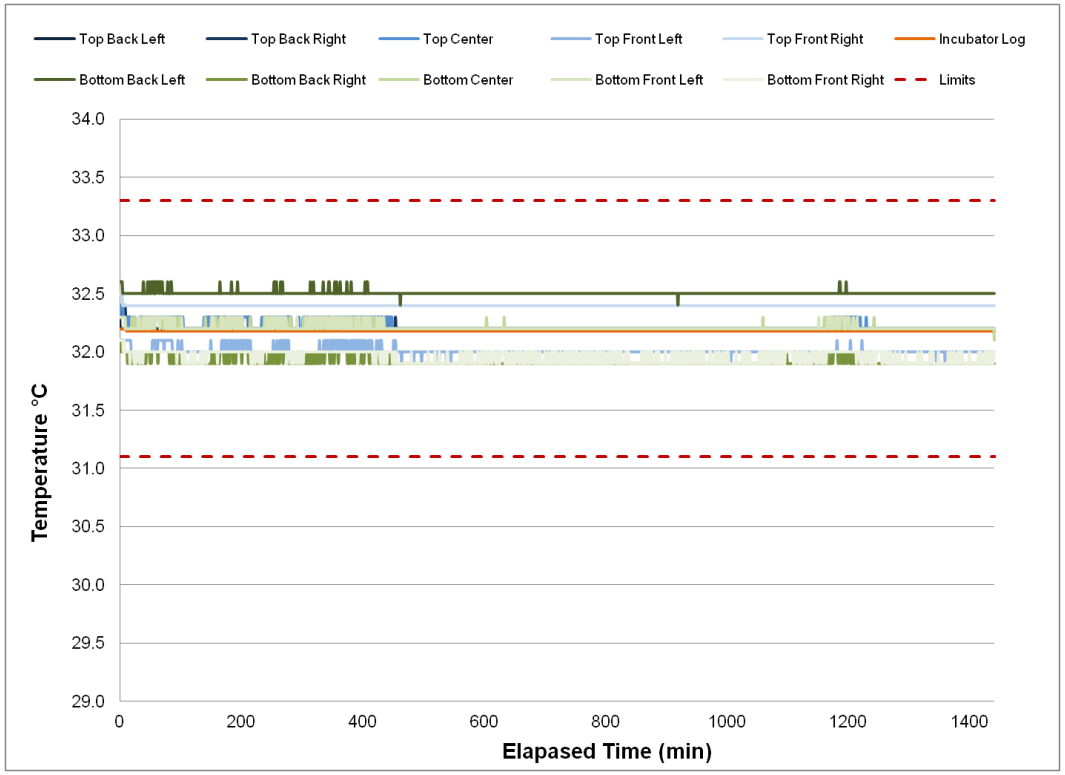


Figure 19. Profile for test chamber temperature-mapping results.

3.4 Analytical Equipment and Procedures

The analytical instrument was an LC-MSMS. The instrument was calibrated with a minimum of five standards ranging from 0.118 to 750 ng/mL. A continuing calibration verification (CCV) sample was included within the range of the calibration curve. A CCV sample was analyzed at least once for every 10 samples.

For the initial verification of the calibration curve, the entire calibration curve and CCV sample were analyzed seven times using standards prepared in acetonitrile, repeating from low to high concentration for each replicate in a single day. The calibration curve replicate results are plotted in Figure 20 and presented in Table 8.

The CCV sample results are plotted in Figure 21 and presented in Table 9. The area responses were analyzed with fit routines to determine the proper weighting scheme as part of the calibration curve development. This development was designed to establish the best representation between the measured response and the analyte concentration.[9,10] For the entire dynamic range, the best fit was found to be described by a quadratic expression with $1/x$ weighting. The weighting was necessary due to the heteroscedastic variability noted in the calibration curve replicates. The unequal variability at the different concentrations indicates that the results violated assumptions required for a linear regression of a nonweighted fit.

The lowest-concentration calibration curve standard (0.118 ng/mL) was higher than the target for five of the seven replicates. Some of the results were outside the target range of ±20%. This was attributed to carryover between analyses. This was not expected to affect testing because smaller dynamic ranges were used, and the individual results from each calibration curve met the accuracy requirements.

During sample analysis, smaller dynamic ranges were used, with a minimum of five levels of calibration standards and a CCV standard. Use of the smaller dynamic range helped to focus the instrument on the concentration of the sample being analyzed. The calibration curve results are plotted in Figure 22 and tabulated in Table 10. The CCV results are plotted in Figure 23 and tabulated in Table 11.

The verification of the calibration curve was repeated with seven additional replicates prepared in acetone, also using the smaller dynamic range. The process was repeated to identify the best match of the calibration solvent with the extraction solvent. The dynamic range was abbreviated, with an upper limit of approximately 100 ng/mL. This compact dynamic range helped to reduce some of the carryover that occurred with higher-concentration samples when the calibration curve ranged up to 700 ng/mL. The shortened range removed the curvature from the upper range of the calibration curve. The calibration curve in acetone was best described by a linear fit with $1/x$ weighting. This abbreviation was only needed for the calibration curve verification procedures, where the samples had a large dynamic range of concentrations that were analyzed simultaneously. Validation testing expanded the range to 500 ng/mL, where the position on the calibration curve was constant for all samples and controlled by dilution level. An abbreviated calibration curve may be useful in future studies if carryover becomes significant.

These results indicated that $1/x$ weighting is appropriate for either the expanded or abbreviated calibration curve range.

Test samples submitted for analysis were diluted volumetrically to be within the calibration curve range. Combinations of class A glassware, class A pipettes, class A volumetric flasks, and gas-tight syringes were used in these dilutions.

The seven replicates for the VX in acetone calibration curve are plotted in Figure 24 and presented in Table 12. The importance and effect of weighting on the calibration curve is demonstrated in Figure 25. The dashed line is a nonweighted linear fit of the data, and the solid line is the 1/*x* weighted linear fit of the data. The data is shown on a log(10) axis to enable visualization of the data. Note that the nonweighted line does not cross the calibration data points at the low concentrations. The unequal variability, greater at the higher concentrations, skewed the data, which caused inaccuracy at the lower concentrations. The calibration curve using the 1/*x* weighting better represented the data. The CCV data points for the seven additional calibration curve verification replicates in acetone are plotted in Figure 26 and presented in Table 13.

The reporting requirement was a table of the prepared standards that included the raw integrated area, calculated concentration, and percent recovery.

The minimum requirements for analytical equipment accuracy were that measurements had to be within 20% of the target for each standard, within 15% of the target for the first CCV sample, and within 10% of the initial CCV response for subsequent CCV samples.

Tables 8–13 fulfill the reporting requirements for the analytical system. The required standards for verification were met for all calibration curve and CCV data points. The validation testing included the use of the shortened calibration curve range, acetone as the solvent for calibration standards, and a linear fit with 1/*x* weighting.

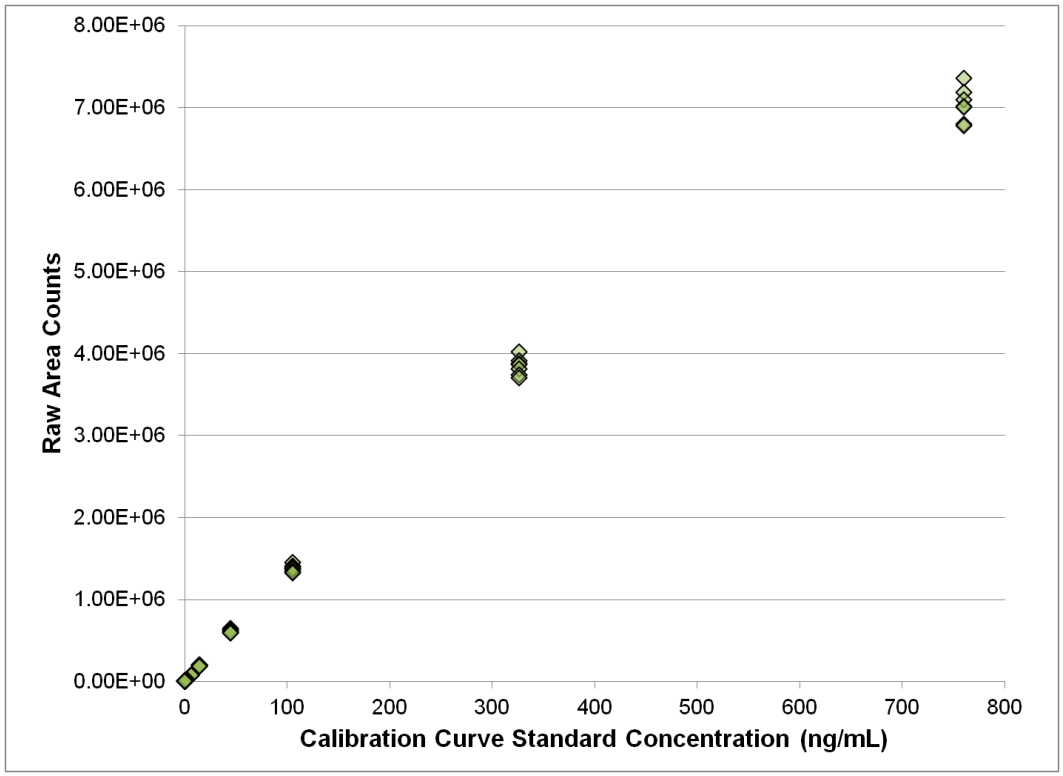


Figure 20. Verification of calibration curve with seven replicates: acetonitrile.

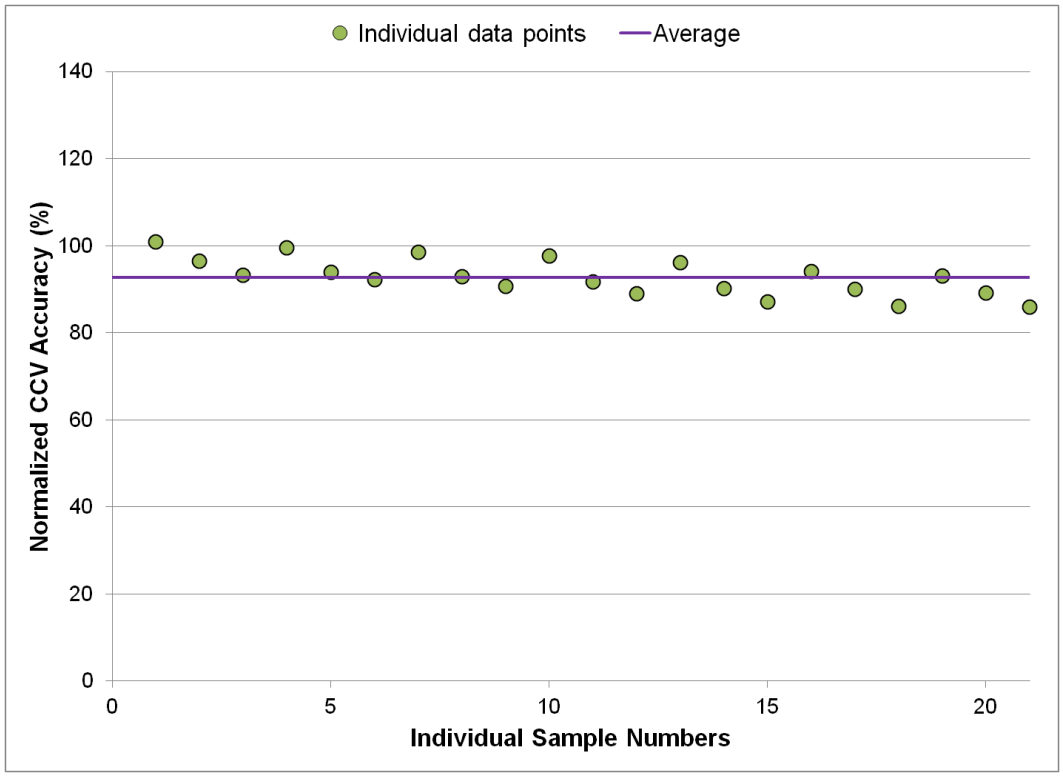


Figure 21. Individual CCV results from initial seven calibration curve replicates: acetonitrile.

Table 8. Calibration Curve Verification Results: Acetonitrile

Target (ng/mL)	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Average Accuracy (%)		Target (ng/mL)	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Average Accuracy (%)
0.118	3,500	0.14	114.4	113.9		14.5	206,867	15.04	103.7	98.8
	3,669	0.15	124.9				202,263	14.70	101.4	
	3,733	0.15	128.8				198,261	14.41	99.4	
	3,682	0.15	125.7				197,885	14.38	99.2	
	3,440	0.13	110.8				195,583	14.21	98.0	
	3,213	0.11	96.7				189,784	13.78	95.0	
	3,205	0.11	96.2				189,942	13.79	95.1	
0.275	5,661	0.29	106.3	104.6		44.9	648,298	48.08	107.1	101.8
	5,764	0.30	109.1				632,527	46.89	104.4	
	5,862	0.31	111.7				621,872	46.08	102.6	
	5,711	0.30	107.7				618,902	45.85	102.1	
	5,608	0.29	104.9				605,880	44.87	99.9	
	5,356	0.27	98.3				598,477	44.31	98.7	
	5,209	0.26	94.4				594,909	44.04	98.1	
0.855	13,226	0.84	98.7	93.9		105	1,450,545	110.78	105.5	100.2
	13,157	0.84	98.1				1,407,639	107.33	102.2	
	13,012	0.83	96.8				1,388,159	105.77	100.7	
	12,685	0.80	94.1				1,380,021	105.12	100.1	
	12,386	0.78	91.5				1,373,188	104.57	99.6	
	12,199	0.77	89.9				1,341,646	102.05	97.2	
	12,024	0.76	88.4				1,329,677	101.10	96.3	
1.99	28,807	1.98	99.5	94.7		326	4,020,001	343.17	105.3	99.9
	28,325	1.94	97.7				3,911,767	332.07	101.9	
	27,883	1.91	96.1				3,875,163	328.35	100.7	
	27,589	1.89	95.0				3,865,098	327.33	100.4	
	26,913	1.84	92.5				3,818,635	322.64	99.0	
	26,742	1.83	91.9				3,738,371	314.59	96.5	
	26,238	1.79	90.1				3,707,801	311.55	95.6	
6.2	83,595	5.98	96.5	92.0		760	7,355,416	834.09	109.7	100.3
	81,526	5.83	94.1				7,188,364	793.73	104.4	
	81,111	5.80	93.6				7,100,883	774.25	101.9	
	79,654	5.69	91.8				7,014,343	755.90	99.5	
	79,310	5.67	91.4				7,004,298	753.82	99.2	
	76,844	5.49	88.5				6,798,353	713.33	93.9	
	76,679	5.48	88.3				6,781,391	710.15	93.4	

Table 9. CCV Results: Acetonitrile

Target (ng/mL)	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Average Accuracy (%)
1.83	25,060	1.71	93.2	89.1
	24,785	1.69	92.1	
	24,397	1.66	90.6	
	23,995	1.63	89.0	
	23,520	1.59	87.1	
	23,250	1.57	86.0	
	23,209	1.57	85.9	
	23,995	1.63	89.0	
17.1	226,434	16.49	96.4	92.0
	220,614	16.06	93.9	
	218,399	15.89	92.9	
	215,660	15.69	91.8	
	211,955	15.42	90.2	
	211,556	15.39	90.0	
	209,382	15.23	89.0	
160	2,064,229	161.38	100.9	97.1
	2,039,908	159.33	99.6	
	2,019,496	157.61	98.5	
	2,003,363	156.25	97.7	
	1,975,090	153.87	96.2	
	1,934,875	150.5	94.1	
	1,915,048	148.85	93.0	

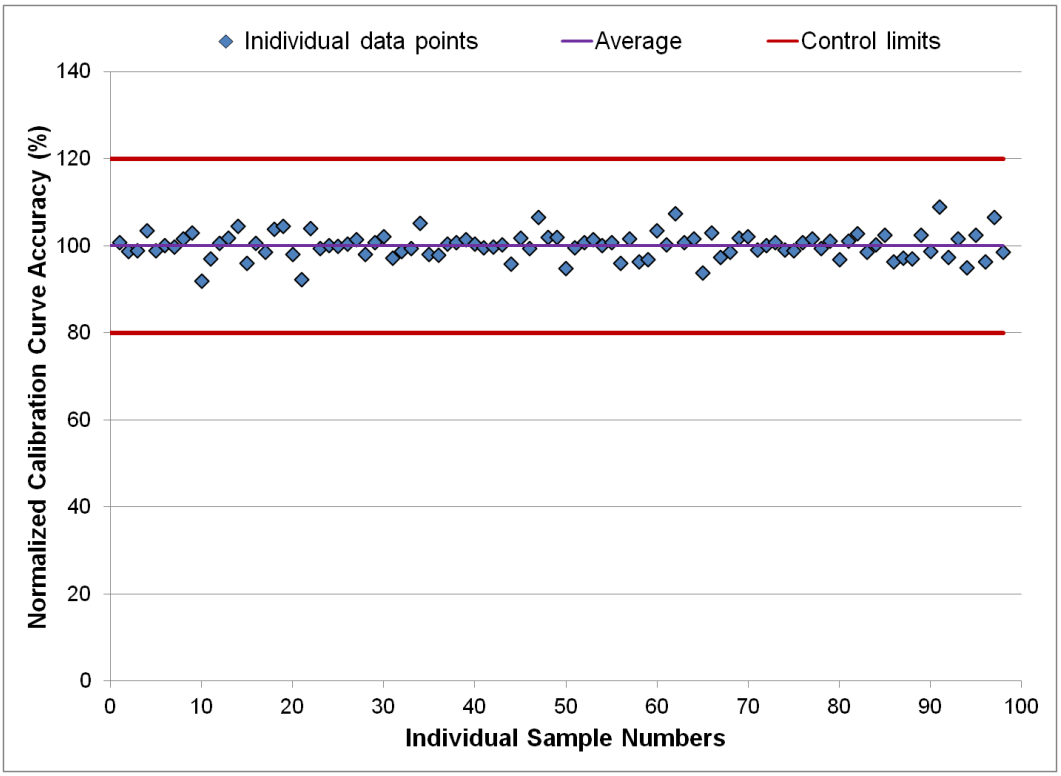


Figure 22. Individual accuracy results for calibration curve standards used during verification testing.

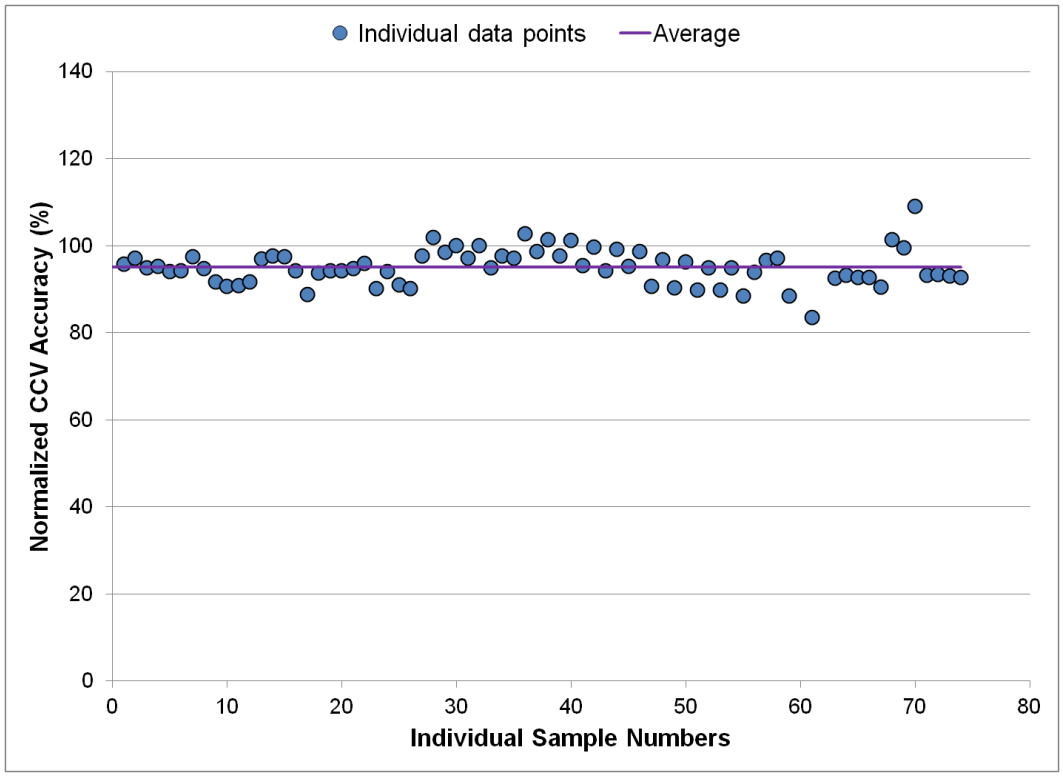


Figure 23. Individual accuracy results for CCV standards used during verification testing.

Table 10. Calibration Curve Results for Each Verification Test Sample Analytical Analysis

Target (ng/mL)	Test	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Target (ng/mL)	Test	Raw Response	Final Conc. (ng/mL)	Accuracy (%)
0.118	D	692	0.12	99.4	14.5	K	65,222	14.02	96.7
0.275	D	1,234	0.29	103.9		L	33,536	14.07	97.0
	K	8,450	0.28	100.1		L	122,034	13.76	94.9
	L	752	0.27	97.3	38.4	C	27,099	39.00	101.7
	L	2,640	0.27	98.4	44.9	D	145,991	44.21	98.5
0.855	D	2,853	0.79	92.2		D	25,884	45.48	101.3
	I	1,030	0.86	100.1		D	25,601	44.64	99.4
	I	1,175	0.87	101.9		I	47,027	45.55	101.5
	I	1,135	0.87	101.6		I	48,710	45.67	101.7
	I	1,268	0.87	101.6		I	52,218	45.51	101.4
	K	10,878	0.84	98.5		I	52,462	46.44	103.4
	L	2,340	0.93	108.9		J	20,485	45.81	102.0
	L	8,436	0.91	106.4		K	26,411	45.19	100.7
1.94	L	4,693	1.92	98.7		K	177,736	45.35	101.0
	C	1,510	2.00	100.6		L	102,033	43.61	97.1
	D	6,593	1.95	97.9		L	379,631	45.59	101.6
	I	2,190	1.98	99.7	105	A	54,738	103.78	98.8
	I	2,249	1.88	94.7		B	56,893	106.64	101.6
	I	2,324	1.91	95.9		C	73,052	105.50	100.5
	I	2,386	1.86	93.7		D	56,994	105.37	100.4
	K	16,008	2.04	102.7		D	56,147	103.52	98.6
	L	17,545	1.92	96.4		I	109,037	105.80	100.8
6.2	A	3,313	6.12	98.8		I	110,961	104.34	99.4
	B	3,522	5.70	91.9		I	121,294	105.82	100.8
	C	4,236	5.90	95.9		I	118,505	105.24	100.2
	D	21,214	6.47	104.4		J	46,720	106.79	101.7
	D	5,087	6.24	100.7		K	58,783	103.71	98.8
	D	5,013	6.08	98.0		L	228,954	101.17	96.4
	I	6,501	6.17	99.6	326	A	161,884	321.41	98.6
	I	7,256	6.60	106.5		B	165,628	325.11	99.7
	I	7,288	6.25	100.8		C	218,395	315.90	96.9
	I	7,766	6.65	107.3		D	164,590	325.29	99.8
	J	3,173	6.21	100.1		D	157,722	316.49	97.1
	K	4,316	6.16	99.3		I	328,345	318.89	97.8
	K	33,730	6.26	101.0		I	336,627	326.93	100.3
	L	15,267	6.35	102.5		I	331,556	312.23	95.8
	L	57,201	6.35	102.4		I	372,388	324.59	99.6
14.5	A	8,068	14.99	103.4		I	352,811	313.82	96.3
	B	8,481	14.92	102.9		I	354,465	315.29	96.7
	C	10,582	15.10	104.4		J	134,828	320.91	98.4
	D	49,097	15.04	103.7		K	171,932	322.77	99.0
	D	9,326	14.19	97.9		L	653,571	333.57	102.3
	D	9,949	15.24	105.1	760	A	348,740	765.84	100.8
	I	15,113	14.54	100.3		B	354,580	760.16	100.0
	I	15,928	14.78	101.9		D	346,575	759.94	100.0
	I	16,733	14.50	100.0		D	329,190	775.61	102.1
	I	16,706	14.61	100.8		J	288,855	738.97	97.2
	J	6,753	14.36	99.0		K	361,917	765.40	100.7
	K	92,016	14.74	101.6	2,361	J	669,150	2431.35	103.0

Table 11. CCV Sample Results for Each Analytical Analysis

Target (ng/mL)	Test	Raw Response	Final Conc. (ng/mL)	Accuracy (%)
1.83	D	5,547	1.63	88.8
17.1	D	52,583	16.1	94.2
		52,409	16.05	93.8
	I	18,090	17.43	101.9
		17,752	17.1	100.0
		17,754	17.11	100.0
		17,975	16.71	97.7
		18,907	17.58	102.8
		18,655	17.35	101.4
		19,936	17.3	101.2
		19,635	17.04	99.6
		19,349	16.96	99.2
		19,232	16.86	98.6
	K	69,539	15.12	88.4
		66,200	14.27	83.5
	L	140,679	15.93	93.2
		141,077	15.98	93.4
		140,569	15.92	93.1
		140,053	15.86	92.7
160	A	80,008	153.27	95.8
		81,070	155.37	97.1
		79,274	151.82	94.9
		79,530	152.32	95.2
		78,542	150.37	94.0
		78,800	150.88	94.3
	B	82,198	155.86	97.4
		80,048	151.65	94.8
		77,546	146.75	91.7
		76,708	145.11	90.7
		76,857	145.4	90.9
		77,498	146.65	91.7
	C	107,372	155.2	97.0
		108,096	156.3	97.7
		107,890	156	97.5
	D	80,121	150.89	94.3
		80,135	150.92	94.3

Target (ng/mL)	Test	Raw Response	Final Conc. (ng/mL)	Accuracy (%)
160	D	80,429	151.5	94.7
		81,366	153.37	95.9
		76,618	144.18	90.1
		79,737	150.47	94.0
		77,349	145.65	91.0
		76,618	144.18	90.1
	I	161,004	156.29	97.7
		162,252	157.51	98.4
		160,168	155.48	97.2
		161,464	151.93	95.0
		165,258	155.51	97.2
		167,784	157.89	98.7
		178,954	156.13	97.6
		174,890	152.58	95.4
		169,734	150.84	94.3
		171,474	152.39	95.2
	J	67,057	154.89	96.8
		66,658	153.94	96.2
		65,796	151.89	94.9
		64,885	151.89	94.9
		65,155	150.36	94.0
	K	86,275	154.75	96.7
		86,692	155.53	97.2
	L	325,739	147.95	92.5
		328,046	149.1	93.2
		326,342	148.25	92.7
		326,332	148.24	92.7
		319,593	144.89	90.6
		353,854	162.08	101.3
		348,383	159.31	99.6
		378,222	174.54	109.1
1,488	J	470,638	1347.97	90.6
		469,496	1343.54	90.3
		467,974	1337.64	89.9
		456,125	1337.64	89.9
		462,156	1315.26	88.4

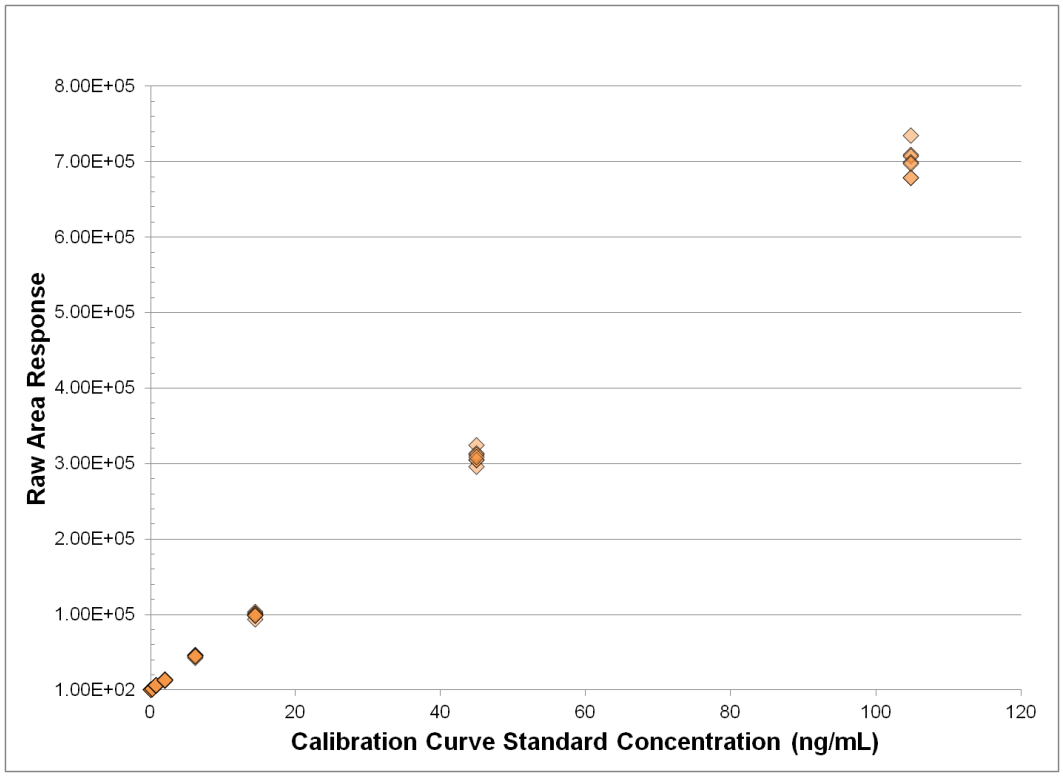


Figure 24. Verification of calibration curve with seven replicates: acetone calibration solvent.

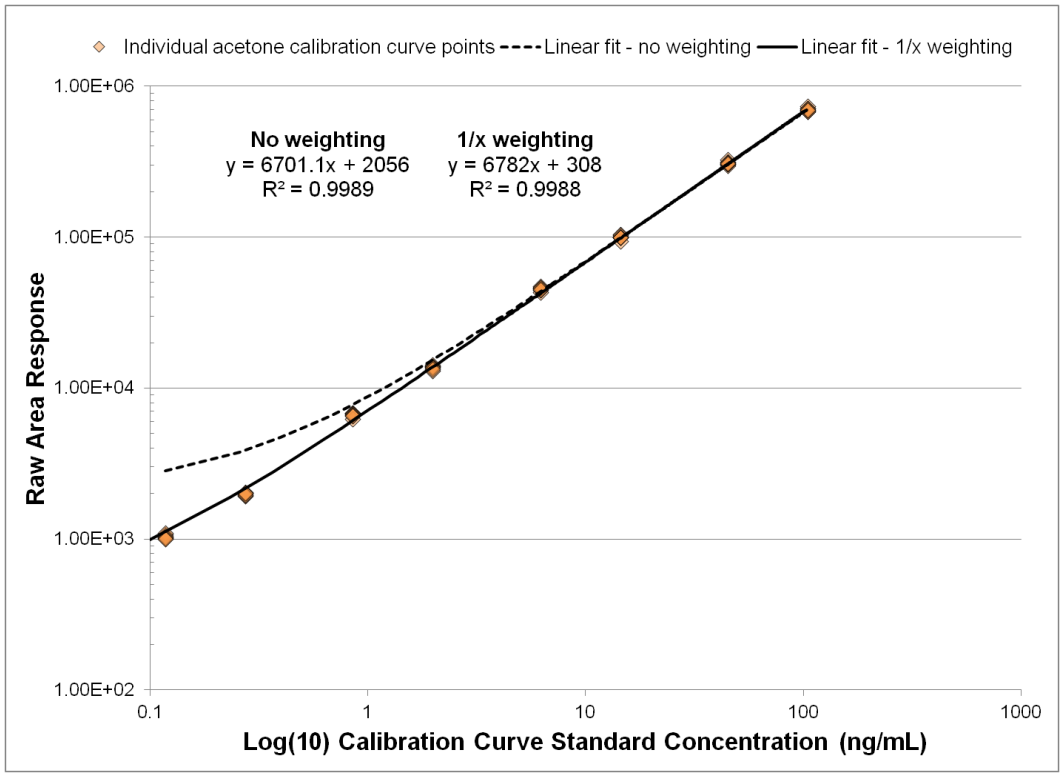


Figure 25. Effect of weighting versus nonweighting on calibration curve performance.

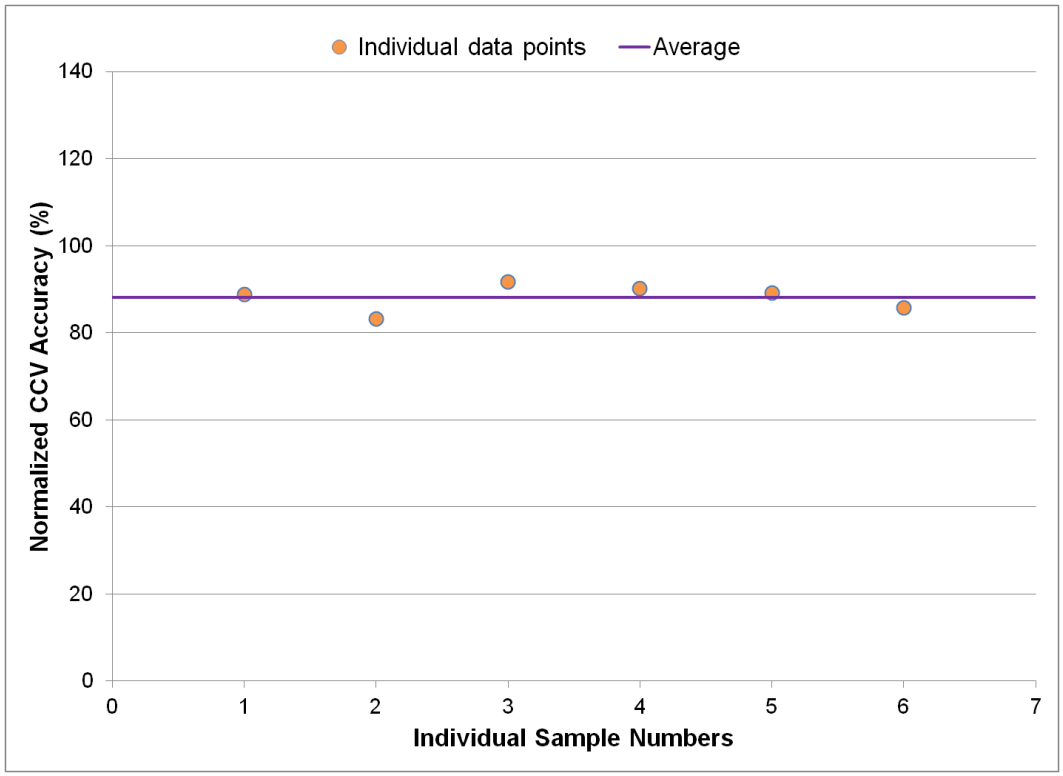


Figure 26. Individual CCV results from seven calibration curve replicates: acetone calibration solvent.

Table 12. Calibration Curve Verification Results: Acetone

Target (ng/mL)	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Average Accuracy (%)	Target (ng/mL)	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Average Accuracy (%)
0.118	1,004	0.11	94.6	98.9	6.2	43,030	5.92	95.4	101.2
	1,008	0.11	94.9			44,878	6.17	99.5	
	1,026	0.11	97.1			45,235	6.22	100.3	
	1,030	0.12	97.5			46,157	6.35	102.4	
	1,050	0.12	99.9			46,238	6.36	102.6	
	1,075	0.12	102.8			46,408	6.38	102.9	
	1,098	0.12	105.5			47,399	6.52	105.1	
0.275	1,928	0.24	86.9	90.1	14.5	94,573	13.03	89.9	95.1
	1,950	0.24	88.0			99,221	13.68	94.3	
	1,964	0.24	88.8			99,428	13.7	94.5	
	2,015	0.25	91.3			99,477	13.71	94.6	
	2,020	0.25	91.6			100,947	13.91	96.0	
	2,020	0.25	91.6			102,475	14.13	97.4	
	2,035	0.25	92.3			104,189	14.36	99.1	
0.855	6,251	0.84	97.8	104.1	44.9	296,482	40.92	91.1	95.2
	6,542	0.88	102.5			305,091	42.11	93.8	
	6,611	0.89	103.6			305,680	42.19	94.0	
	6,704	0.9	105.1			309,203	42.67	95.0	
	6,728	0.9	105.5			312,209	43.09	96.0	
	6,807	0.91	106.8			314,032	43.34	96.5	
	6,844	0.92	107.4			325,112	44.87	99.9	
1.99	13,041	1.77	89.1	94.0	104.8	735,093	101.49	96.8	92.4
	13,385	1.82	91.5			678,836	93.72	89.4	
	13,636	1.86	93.3			679,383	93.8	89.5	
	13,858	1.89	94.8			698,284	96.41	92.0	
	13,976	1.9	95.6			699,578	96.59	92.2	
	14,013	1.91	95.9			708,095	97.76	93.3	
	14,255	1.94	97.6			709,542	97.96	93.5	

Table 13. CCV Results: Acetone

Target (ng/mL)	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Average Accuracy (%)
17.1	110,147	15.18	88.8	88.1
	103,182	14.22	83.2	
	113,698	15.67	91.7	
	111,774	15.41	90.1	
	110,636	15.25	89.2	
	106,355	14.66	85.7	

3.5 Agent Application Proficiency

Two operators spiked eight PTFE disks with six 1 µL drops of VX, and the disks were extracted in 20 mL of acetonitrile. The theoretical mass was 5580 µg/sample, accounting for the 93% agent purity from the CoA. The results are shown in Table 14.

The proficiency reporting requirements included a table of the operator number that provided target total mass, measured total mass, percent recovery for each sample, average percent recovery, standard deviation, and relative standard deviation (i.e., standard deviation divided by average percent recovery). If operator proficiency had previously been demonstrated, the data and appropriate citations had to be provided.

The minimum requirement for operator agent application proficiency was an accuracy value that was within 15% of the target value for each sample in the verification set, from a minimum of eight replicates.

Table 14 fulfills the reporting requirement for agent application proficiency. The target requirements were met for all samples, and both operators demonstrated agent application proficiency. These results demonstrate that the agent had not degraded, that operator bias was negligible, and analytical bias was negligible.[9]

Table 14. Operator Proficiency Test Results

Operator	Replicate	Mass Deposited (µg)	Percent of Target (%)	Average (%)	StDev (%)	RSD (%)
1	1	5580	100.0	103.2	2.4	2.3
	2	5827	104.4			
	3	5801	104.0			
	4	5830	104.5			
	5	5815	104.2			
	6	5938	106.4			
	7	5734	102.8			
	8	5554	99.5			
2	1	5637	101.0	102.5	1.0	1.0
	2	5759	103.2			
	3	5688	101.9			
	4	5691	102.0			
	5	5728	102.7			
	6	5803	104.0			
	7	5774	103.5			
	8	5681	101.8			

3.6 Contact Weight Requirements

The contact weights provided the necessary contact between the contaminated swatch and the underlying sorption pad. Each contact weight had five critical parameters: construction material, weight numbering, mass, contact area diameter, and contact-area nub height. Each weight produced a pressure equivalent to 1 psi. A diagram of a contact weight is shown in Figure 1.

The weights were made of type 316 stainless steel, and each was numbered with a three-digit code, from 001 through 042. The mass of each weight was measured on a NIST-traceable calibrated balance. The spatial dimensions of the contact area were measured using a calibrated micrometer. The calibration information for these tools is provided in Section 5.6.

The individual contact weight measurements are provided in Table 15 and are summarized in Table 16.

The minimum reporting requirements for the contact weights included identification of the construction material and description of the numbering scheme for the weights. For the mass, the scale brand, model number, serial number, calibration date, and calibration expiration date were provided along with the mass of each weight, in grams, to the nearest 5 g. For the spatial dimensions, the measurement tool brand, model number, serial number, calibration date, and calibration expiration date were provided along with the measurements of the contact area diameter and length of the nub, in inches, to the nearest 0.001 in.

The minimum requirements were that the weights be made of stainless steel and individually numbered. The mass target was 453.6 g with a 5 g tolerance. The target diameter was 28.651 mm with a 0.254 mm tolerance. The target nub length was 3.277 mm with a 0.254 mm tolerance.

Tables 15 and 16 fulfill the reporting requirements for the contact weights. The LVAP V&V requirements were met for the contact weights.

Table 15. Individual Contact Weight Measurements

Weight No.	Mass (g)	Diameter (mm)	Nub Length (mm)		Weight No.	Mass (g)	Diameter (mm)	Nub Length (mm)
001	449.0	28.702	3.302		022	449.3	28.727	3.302
002	449.4	28.651	3.175		023	449.2	28.702	3.277
003	449.6	28.753	3.454		024	449.4	28.727	3.175
004	449.3	28.702	3.353		025	448.2	28.702	3.277
005	449.6	28.727	3.302		026	449.5	28.702	3.302
006	449.8	28.753	3.150		027	449.2	28.702	3.302
007	449.6	28.753	3.353		028	449.0	28.727	3.404
008	449.9	28.778	3.277		029	449.5	28.702	3.150
009	449.3	28.778	3.124		030	449.1	28.727	3.251
010	449.2	28.778	3.277		031	449.2	28.702	3.251
011	449.7	28.753	3.277		032	449.3	28.727	3.277
012	449.3	28.727	3.150		033	449.2	28.727	3.327
013	449.6	28.753	3.251		034	448.8	28.753	3.200
014	449.5	28.702	3.327		035	449.3	28.702	3.251
015	449.9	28.702	3.277		036	449.6	28.727	3.531
016	449.6	28.727	3.124		037	449.4	28.702	3.327
017	449.6	28.778	3.150		038	449.2	28.753	3.404
018	449.4	28.702	3.150		039	449.2	28.677	3.302
019	449.5	28.651	3.277		040	449.6	28.702	3.302
020	449.7	28.702	3.378		041	449.0	28.702	3.378
021	449.7	28.727	3.353		042	448.8	28.677	3.378

Table 16. Summary: Contact Weight Measurements

Summary	Mass (g)	Diameter (mm)	Nub Length (mm)
Average	449.4	28.721	3.282
StDev	0.3	0.31	0.092
RSD	0.07%	0.11%	2.79%
Range	448.2–449.9	28.651–28.778	3.124–3.531

3.7 Uptake and Extraction Efficiency Verification: 24 h Time Point

The efficacy reporting requirements included a completed run sheet and the tabulated data of the individual sample concentrations for the DVB extractions and the controls. The efficiency for each sample was compared with the control average, along with the average uptake efficiency, the standard deviation, and the relative standard deviation. The run sheet documented the individual sample identification numbers, sample positions, spike times, solvent addition times, aliquot removal times, and observations.

The method acceptance limits for efficiency required that values had to be within 30% of the target control value for each concentration tested.

3.7.1 Uptake and Extraction Efficiency Verification Goals

The initial goals of uptake and extraction efficiency verification were to document the DVB sorption pad performance for VX with (1) a 24 h contact time, (2) 30 and 60 min extraction times, and (3) 20 mL extraction in a 240 mL jar and 10 mL extraction in a 60 mL jar.

Use of the smaller vessel was envisioned as a means to increase the sensitivity of extracted samples by requiring less solvent and to simultaneously reduce the waste handling of excess acetonitrile and contaminated glass. Two extraction time points were examined to determine whether a benefit was associated with a longer extraction period.

Subsequent goals related to efficiency testing included the following:

- Examine the effect of activation-processed DVB pads;
- Measure the effect of a second extraction in fresh solvent;
- Compare two additional extraction solvents, acetone and methanol; and
- Document the performance of the selected solvent and conditions.

3.7.2 Uptake and Extraction Efficiency Verification Power Statement

A statistical analysis was performed on extraction efficiency and solvent spike data to determine the number of replicates required to measure the mean with a particular tolerance limit. The calculation was dependent on the confidence interval (α), the standard deviation (σ), and $t_{1-\alpha/2}$. The calculation was performed using the following:[8]

$$n = \frac{t^2 \sigma^2}{d^2} \tag{1}$$

where t is $t_{1-\alpha/2}$ for ν degrees of freedom, and d is the allowable tolerance.

The minimum number of samples was calculated for three concentration levels of the spike solvent control and the DVB pad extraction efficiency by measuring the standard deviation for each sample subset, obtaining the t statistic from reference tables, and establishing the tolerance limit. The calculated minimum numbers of samples are shown in Table 17.

Table 17. Minimum Numbers of Replicates Required for Spike Solvent Control and DVB Pad Extraction Efficiency Samples

Sample Type	Spike Solvent			DVB Extraction Efficiency		
	Low Conc.	Medium Conc.	High Conc.	Low Conc.	Medium Conc.	High Conc.
RSD (%)	0.45	0.57	0.85	1.58	0.83	0.55
Tolerance limit (% mean)	2	2	2	2	2	2
Minimum number of samples	1	2	4	5	2	1
Degrees of freedom	2	2	2	4	4	4

From this calculation, the condition with the greatest relative standard deviation was the low-concentration DVB pad extraction efficiency. In that case, a minimum of five replicates was sufficient to have a tolerance about the mean within 2%. The purpose of this calculation was to calculate the number of replicates required to reach a particular tolerance limit, given the past performance standard deviation. It was not a requirement that the extraction efficiency evaluation meet this 2% tolerance limit.

Based on this calculation, five replicates per concentration were sufficient for the efficiency studies.

3.7.3 Uptake and Extraction Efficiency Scope

Because of the competitive nature of the contaminant interaction between the two materials, the uptake potential may vary as a function of sorbent pad and substrate. Obtaining an accurate measurement of contaminant on a surface may present a difficult challenge, as many substrates are sorptive. The measurement may be confounded by the sorption of the contaminant into the substrate, where it is no longer accessible by the sorbent pad. To address this confounding, the PTFE was also analyzed as an independent assessment of the uptake, without the potential confounding effect of DVB extraction efficiency.

The contact efficiency might have also been affected by the contact area of the sorbent pad, contact times, pressures, and contamination levels.

The uptake efficiency verification test only considered contaminant on PTFE as a nonsorptive, nonreacting substrate. A single contact time point (24 h) and pressure (1 psi) were considered for three contamination levels. Two different extraction jar sizes (60 and 240 mL) were characterized, each of which had a different extraction volume (10 and 20 mL, respectively). For each sample, two different extraction times (30 and 60 min) were examined.

The spike volume, deposited as 50 µL, was held constant. The starting concentration solutions were 4, 20, and 100 µg/mL for the 60 mL jar and 8, 40, and 200 µg/mL for the 240 mL jar. These produced target concentrations of 20, 100, and 500 ng/mL, respectively.

The same volume (50 µL) and concentrations were applied to the DVB sorbent pad for the initial extraction efficiency study.

The scope for the uptake and extraction efficiency testing was expanded after completion of the initial scoping work. The efficiencies were not as high as expected; therefore, two additional tests were conducted.

The first additional experiment was a scoping test to examine potential causes for the low extraction performance. Variables included dry versus wet prepared pads, a second extraction in fresh solvent, and solvent choice of acetone versus methanol. Further testing was performed to examine for reaction products. To focus on these parameters, testing was limited to a single contamination concentration, and only extraction efficiency was conducted; uptake efficiency testing was not conducted during this additional scoping test.

The second additional experiment was conducted with acetone and a dry pad at three concentrations. This was a down-selection from the previous scoping experiment. Both uptake and extraction efficiency tests were conducted.

3.7.4 Uptake and Extraction Efficiency Experiments

3.7.4.1 DVB Pad Washing and Activation Steps

The initial plan included no washing or activation of the DVB pads; instead, the pads were to be used in the as-packaged, dry configuration. However, wetted pads were used during the methanol versus acetone extraction efficiency scoping test. These pads were prepared with a series of solvents, ending with water, in accordance with manufacturer's instructions.

3.7.4.2 Preparation of Samples for Uptake Efficiency

Sample preparation included the following procedures:

- The inverted jar lid was used as a platform: the small Petri dish was placed in the middle of the lid, and one PTFE disk was placed in the Petri dish.
- Dilute solution (50 µL) was spiked onto the PTFE disk, and the time was noted on the run sheet.
- Due to the highly variable dry times, all PTFE disks were spiked sequentially, with no additional time between spiking.
- One PTFE disk was not spiked with the solution and served as a negative control.
- The solvent was allowed to evaporate to dryness (approximately 10–30 min). Dryness was indicated when there was no longer a sessile drop on the surface of the PTFE. This time varied depending on the solvent used, exact drop morphology, and underlying substrate morphology. There were no tolerance limits on the drying time; however, the times for spiking and DVB application were noted.
- The PTFE (including the negative control) was covered with a DVB sorbent pad.
- The DVB sorbent pad was covered with a second PTFE disk to prevent the weight from cross-contaminating the DVB pad.
- The weight was applied.
- The glass of the jar was used as a cover and seal.
- Each jar was placed into the incubator test chamber for 24 h.
- The temperature of the incubator test chamber was recorded.

3.7.4.3 Extraction of Uptake DVB Sorbent Pads and PTFE Swatches

During the initial test, the following procedures were performed:

- For the larger 240 mL jar extraction, the extraction jar was filled with 20 mL of acetonitrile.
- For the smaller 60 mL jar extraction, the extraction jar was filled with 10 mL of acetonitrile.
- The DVB was extracted in one jar (either 60 or 240 mL, as appropriate), and the spiked PTFE was extracted in another jar of appropriate volume.
- In preparation for analysis, aliquots were taken at 30 and 60 min intervals and placed in 2 mL autosampler vials.

During the subsequent test with acetone, the following procedures were performed:

- The 60 mL jars were filled with 20 mL of acetone.
- The DVB was extracted in one jar, and the spiked PTFE was extracted in another jar.
- After the initial 30 min extraction time, an aliquot was removed, and the DVB was moved to a fresh jar of solvent for a second extraction of an additional 30 min.
- In preparation for analysis, aliquots were taken and placed in 2 mL autosampler vials.

All extracts were stored at ≤4 °C and analyzed within 14 days.

3.7.4.4 Uptake Efficiency Positive-Control Steps

The purpose of the positive control was to demonstrate that the spiking and extraction processes for the PTFE swatch were within acceptable control limits. This portion of testing was conducted using only the spiked PTFE swatches, and the extraction duration was varied. Procedures for all positive controls included the following:

- The inverted jar lid was used as a platform. A large Petri dish was placed in the middle of the lid, and one PTFE disk was placed in the Petri dish.
- Three of the disks were spiked with 50 µL of the chosen solution. Due to the highly variable dry times, all PTFE disks were spiked sequentially, with no additional time allotted between spiking.
- The solvent was allowed to evaporate to dryness. Dryness was indicated when there was no longer a sessile drop on the surface of the PTFE. This time varied depending on the solvent used, exact drop morphology, and underlying substrate morphology. There were no tolerance limits on the drying time; however, the times for spiking and extraction were noted.
- During the initial test configuration with acetonitrile, as a control for either configuration, the PTFE disk was extracted in the chosen jar size (60 or 240 mL) with the appropriate volume of acetonitrile (10 or 20 mL) for 30 min before the first aliquot was removed. The second aliquot was removed at 60 min.

- During the subsequent test with acetone, the PTFE disk was extracted in 20 mL of acetone in a 60 mL jar for 30 min before an aliquot was removed. These samples served as controls for both the first and second extractions.

All extracts were stored at ≤4 °C and analyzed within 14 days.

3.7.4.5 Extraction Efficiency Steps

During the initial test with acetonitrile, the following steps were performed:

- DVB sorbent pads were placed on the bottoms of 60 and 240 mL glass jars.
- Each DVB pad was spiked with 50 µL of target spiking solution. Spikes were separated by ~2 min to allow time for breakdown and extraction.
- After 24 h, 20 mL of acetonitrile was added to each 240 mL jar, and 10 mL of acetonitrile was added to each 60 mL jar.
- Each DVB pad was extracted for 30 min, and the first aliquot was removed. The second aliquot was removed at 60 min.

For subsequent tests with acetone or methanol, the following steps were performed:

- DVB sorbent pads were placed on the bottoms of 60 mL glass jars.
- Each DVB pad was spiked with 50 µL of target spiking solution. Spikes were separated by ~1 min to allow time for breakdown and extraction.
- After 24 h, 20 mL of solvent was added to each jar.
- Each DVB pad was extracted for 30 min, and the first aliquot was removed.
- Each DVB pad was transferred (with a clean pair of disposable forceps) to a second jar already filled with 20 mL of fresh solvent. The pad was extracted for another 30 min, and a second aliquot was removed.

All extracts were stored at ≤4 °C and analyzed within 14 days.

3.7.4.6 Extraction Efficiency Positive-Control Steps

During the initial test with acetonitrile, the following steps were performed:

- Glass jars (240 mL) containing 20 mL of acetonitrile were spiked with 50 µL of a target standard solution. One solution was added to each jar, and five replicates were prepared per solution.
- Glass jars (60 mL) containing 10 mL of acetonitrile were spiked with 50 µL of a target standard solution. One solution was added to each jar, and five replicates were prepared per solution.
- Spikes were separated by ~2 min to allow for processing time.
- The first aliquot was removed after 30 min, and the second aliquot was removed at 60 min.

For the subsequent tests with acetone or methanol, the following steps were performed:

- Glass jars (60 mL) containing 20 mL of solvent were spiked with 50 µL of target standard solution.

- Spikes were separated by ~1 min to allow for processing time.
- Aliquots were removed at 30 min.

All extracts were stored at ≤4 °C and analyzed within 14 days.

3.7.5 Uptake and Extraction Efficiency Verification Calculations

The target sample concentrations are shown in Table 18 for the initial testing with acetonitrile and Table 19 for the subsequent testing with acetone.

Table 18. Target Extraction Concentrations for Initial Uptake and Extraction Efficiency Verifications with Acetonitrile

Variable	**60 mL Configuration**			**240 mL Configuration**		
Spike volume (µL)	50	50	50	50	50	50
Concentration of spiking solution (µg/mL)	4	20	100	8	40	200
Mass applied (µg)	0.2	1.0	5.0	0.4	2.0	10.0
Extraction volume (mL)	10	10	10	20	20	20
Theoretical concentration (ng/mL)	20	100	500	20	100	500

Table 19. Target Extraction Concentrations for Subsequent Uptake and Extraction Efficiency Verifications with Acetone

Variable	**60 mL Configuration**		
Spike volume (µL)	50	50	50
Concentration of spiking solution (µg/mL)	12	80	360
Mass applied (µg)	0.6	4.0	18.0
Extraction volume (mL)	20	20	20
Theoretical concentration (ng/mL)	30	200	900

Calculating the uptake efficiency required the comparison of the extracted sample to a known standard. The measured concentration for each uptake efficiency sample and spiked PTFE sample was multiplied by the solvent volume to produce the total mass of contaminant recovered. The total masses of the spiked PTFE samples were averaged, producing the known standard target of analysis in the absence of the sorbent layer. The extracted mass for each uptake efficiency sample was divided by the average of the spiked PTFE samples to yield the uptake efficiency percentage for that particular sample. The results for all of the extraction efficiency samples were averaged to calculate the overall uptake efficiency performance for the sorbent. The calculation for uptake efficiency is as follows:

$$UE_{DVB} = \frac{m_u}{\bar{m}_{PTFE}} \times 100 \quad (2)$$

where *UE* is the uptake efficiency, m_u is the extracted mass for each uptake efficiency sample, and $\bar{m}_{PTFE}$ is the average of the spiked PTFE samples.

The uptake efficiency can also be calculated from the PTFE sample extraction. The extracted mass for each uptake efficiency PTFE sample was divided by the average of the spiked PTFE samples. This result was the uptake efficiency percentage for that particular sample. The results for all of the extraction efficiency samples were averaged to calculate the overall uptake efficiency performance for the sorbent. Here, a higher uptake efficiency was indicated by a lower measured mass remaining on the initial PTFE sample, as follows:

$$UE_{PTFE} = \left(1 - \frac{m_{UP}}{\bar{m}_{PTFE}}\right) \times 100 \quad (3)$$

where m_{UP} is the uptake efficiency for one PTFE sample.

Calculating the extraction efficiency required the comparison of an extracted sample to a theoretically calculated value. The measured concentration for each extraction efficiency sample and solvent spike was multiplied by the solvent volume to produce the total mass of contaminant recovered. The total masses of the spiked solvent samples were averaged, producing the known standard target of analysis in the absence of the sorbent layer. The extracted mass for each extraction efficiency sample was divided by the average of the spiked samples to yield the extraction efficiency percentage for that particular sample. The results for all of the extraction efficiency samples were averaged to calculate the overall extraction efficiency performance for the sorbent. The calculation for a single sample extraction efficiency is as follows:

$$EE = \frac{m_e}{\bar{m}_{spike}} \times 100 \quad (4)$$

where *EE* is the extraction efficiency, m_e is the extracted mass for one extraction efficiency sample, and $\bar{m}_{spike}$ is the average of the spiked samples.

3.7.6 Uptake and Extraction Efficiency Results

3.7.6.1 Initial Uptake and Extraction Efficiency Results with Acetonitrile

The summary results for the initial verification test with acetonitrile are provided in Table 20 for extraction efficiency and Table 21 for uptake efficiency. Individual sample results for extraction efficiencies obtained from the 20 and 10 mL acetonitrile extractions are shown in Tables 22 and 23. Individual results for the 20 and 10 mL acetonitrile extractions are provided in Tables 24 and 25.

Table 20. Summary Initial Extraction Efficiency Results: Acetonitrile

Extraction Volume (mL)	Target Mass (ng)	30 min Extraction			60 min Extraction		
		Average (%)	StDev (%)	RSD (%)	Average (%)	StDev (%)	RSD (%)
10	200	51.4	4.7	9.1	50.3	5.0	9.9
	1,000	58.7	2.2	3.7	60.4	2.1	3.5
	5,000	67.5	2.5	3.7	67.3	1.6	2.4
20	400	62.6	2.7	4.3	57.6	2.5	4.3
	2,000	67.8	1.6	2.4	68.0	0.8	1.2
	10,000	76.8	2.6	3.4	76.1	1.4	1.9

Table 21. Summary Initial Uptake Efficiency Results: Acetonitrile

Extraction Volume (mL)	Sample Type	Target Mass (ng)	30 min Extraction			60 min Extraction		
			Average (%)	StDev (%)	RSD (%)	Average (%)	StDev (%)	RSD (%)
10	DVB	200	84.9	4.9	5.7	73.8	2.7	3.6
		1,000	71.7	6.6	9.2	63.9	1.9	3.0
		5,000	65.3	9.5	14.5	73.4	2.8	3.8
	PTFE	200	32.2	38.1	118.6	>99.9	n/a	n/a
		1,000	>99.9	n/a	n/a	>99.9	n/a	n/a
		5,000	>99.9	n/a	n/a	>99.9	n/a	n/a
20	DVB	400	107.7	9.4	8.7	107.4	9.3	8.7
		2,000	77.5	4.0	5.1	76.4	4.4	5.8
		10,000	80.7	2.5	3.1	80.9	1.7	2.1
	PTFE	400	97.8	2.9	3.0	97.7	3.1	3.1
		2,000	99.4	0.4	0.4	99.4	0.5	0.5
		10,000	99.0	1.0	1.0	98.9	1.0	1.0

n/a, not applicable.

Table 22. Extraction Efficiency Results: 20 mL Acetonitrile Extraction

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average (%)	
		30 min	60 min	30 min	60 min	30 min	60 min
DVB extraction	400	260.6	254.0	63.7	59.0	62.6	57.6
		260.5	252.7	63.7	58.7		
		241.4	234.5	59.0	54.5		
		257.5	248.7	62.9	57.8		
		245.9	236.6	60.1	55.0		
		271.6	262.1	66.4	60.9		
	2,000	1,270	1,283	65.5	67.2	67.8	68.0
		1,345	1,310	69.4	68.5		
		1,327	1,314	68.5	68.8		
		1,315	1,290	67.8	67.5		
	10,000	7,349	7,205	79.8	78.2	76.7	76.1
		6,909	6,959	75.0	75.5		
		6,756	6,843	73.3	74.3		
		7,255	7,045	78.7	76.5		
		7,108	7,008	77.1	76.1		
Solvent spike control	400	408.5	426.3	n/a			
		407.4	430.8				
		411.7	434.6				
	2,000	1,950	1,918				
		1,925	1,893				
		1,941	1,923				
	10,000	9,126	9,195				
		9,229	9,209				
		9,286	9,247				

n/a, not applicable.

Table 23. Extraction Efficiency Results: 10 mL Acetonitrile Extraction

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average (%)	
		30 min	60 min	30 min	60 min	30 min	60 min
DVB extraction	200	108.5	102.6	56.1	54.3	51.4	50.3
		104.3	99.3	54.0	52.5		
		84.8	79.4	43.9	42.0		
		98.0	93.6	50.7	49.5		
		101.5	101.0	52.5	53.4		
	1000	562.6	544.7	59.7	59.1	58.7	60.4
		538.8	533.4	57.2	57.9		
		538.2	554.9	57.1	60.2		
		586.1	582.4	62.2	63.2		
		543.4	570.1	57.6	61.8		
	5000	3234	3159	68.5	66.9	67.5	67.3
		3264	3198	69.1	67.7		
		2983	3062	63.1	64.8		
		3193	3223	67.6	68.2		
		3263	3257	69.1	69.0		
Solvent spike control	200	193.6	189.0	n/a			
		192.8	188.8				
		193.7	189.5				
	1000	953.4	930.3				
		945.6	923.0				
		929.2	912.3				
	5000	4736	4866				
		4692	4684				
		4744	4857				

n/a, not applicable.

Table 24. Uptake Efficiency Results: 20 mL Acetonitrile Extraction

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average (%)	
		30 min	60 min	30 min	60 min	30 min	60 min
DVB extraction	400	286.9	274.3	114.4	109.4	107.7	107.4
		249.3	253.2	99.4	101.0		
		241.2	239.0	96.2	95.3		
		293.9	297.1	117.2	118.5		
		279.0	283.5	111.3	113.1		
	2,000	1,456	1,431	81.1	79.7	77.5	76.4
		1,360	1,262	75.8	70.4		
		1,309	1,443	72.9	80.4		
		1,474	1,405	82.1	78.3		
		1,350	1,310	75.2	73.0		
	10,000	7,336	7,190	81.0	79.4	80.7	80.9
		7,649	7,596	84.5	83.9		
		7,344	7,244	81.1	80.0		
		7,169	7,302	79.2	80.6		
		7,058	7,318	77.9	80.8		
PTFE sample	400	18.1	19.2	92.8	92.4	97.8	97.7
		2.4	2.5	99.0	99.0		
		4.8	5.0	98.1	98.0		
		0.9	0.9	99.6	99.6		
		0.9	1.0	99.6	99.6		
	2,000	7.4	11.1	99.6	99.4	99.4	99.4
		23.6	26.6	98.7	98.5		
		8.1	5.5	99.6	99.7		
		9.7	8.4	99.5	99.5		
		5.8	4.3	99.7	99.8		
	10,000	15.6	19.4	99.1	98.9	99.0	98.9
		49.6	51.6	97.2	97.1		
		11.3	7.9	99.4	99.6		
		7.5	10.2	99.6	99.4		
		9.5	10.8	99.5	99.4		
PTFE control	400	281.2	280.0	n/a			
		340.1	131.5				
		131.0	340.4				
	2,000	1,821	1,817				
		1,822	1,790				
		1,740	1,717				
	10,000	9,052	9,022				
		9,153	9,101				
		8,962	8,996				

n/a, not applicable.

Table 25. Uptake Efficiency Results: 10 mL Acetonitrile Extraction

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average (%)	
		30 min	60 min	30 min	60 min	30 min	60 min
DVB extraction	200	119.2	106.4	81.2	72.4	84.9	73.8
		136.3	114.9	92.8	78.2		
		124.3	108.7	84.6	74.0		
		125.1	107.8	85.2	73.4		
		118.4	104.5	80.6	71.1		
	2000	610.6	542.6	74.3	66.0	71.7	63.9
		617.8	526.3	75.1	64.0		
		619.6	538.8	75.4	65.5		
		606.8	517.5	73.8	62.9		
		492.8	503.6	59.9	61.2		
	5000	3441	3211	76.7	71.6	65.3	73.4
		2399	3478	53.5	77.5		
		3244	3188	72.3	71.1		
		2637	3363	58.8	75.0		
		2936	3217	65.4	71.7		
PTFE sample	200	BQL	BQL	>99.9	>99.9	32.2	>99.9
		134.9	*	8.2	*		
		122.3	*	16.8	*		
		122.2	*	16.8	*		
		118.9	*	19.1	*		
	1000	BQL	BQL	>99.9	>99.9	>99.9	>99.9
		BQL	BQL	>99.9	>99.9		
		BQL	BQL	>99.9	>99.9		
		BQL	BQL	>99.9	>99.9		
		BQL	BQL	>99.9	>99.9		
	5000	BQL	BQL	>99.9	>99.9	>99.9	>99.9
		BQL	BQL	>99.9	>99.9		
		BQL	BQL	>99.9	>99.9		
		BQL	BQL	>99.9	>99.9		
		BQL	BQL	>99.9	>99.9		
PTFE control	200	147.3	143.4	n/a			
		142.3	139.6				
		151.1	149.4				
	1000	813.4	787.4				
		836.2	838.4				
		816.9	820.6				
	5000	4317	4325				
		4614	4600				
		4529	4551				

*Outliers with attribution: potentially mislabeled samples; cf. Section 3.7.7.
BQL, below quantification limit.
n/a, not applicable.

3.7.6.2 Additional Scoping Extraction Efficiency Test

The summary results for the additional extraction efficiency scoping test with methanol and acetone are shown in Table 26. The individual sample results are provided in Table 27.

Table 26. Summary of Extraction Efficiency Additional Scoping Test

Solvent	Condition	1st Extraction			2nd Extraction			Total (%)
		Average (%)	StDev (%)	RSD (%)	Average (%)	StDev (%)	RSD (%)	
Methanol	Dry	84.7	2.7	3.2	9.6	1.4	14.6	94.3
	Wet	75.4	7.0	9.3	10.8	1.4	13.3	86.2
Acetone	Dry	86.3	1.1	1.3	4.8	0.3	6.1	91.1
	Wet	80.4	3.6	4.5	5.1	0.6	10.9	85.5

Table 27. Extraction Efficiency Additional Scoping Test Results

Sample Type	Solvent	Cond.	Mass Recovered (ng)		Efficiency (%)		Average Efficiency (%)	
			1st Extraction	2nd Extraction	1st Extraction	2nd Extraction	1st Extraction	2nd Extraction
DVB extraction	Methanol	Dry	920.7	118.4	85.9	11.0	84.7	9.6
			919.8	99.3	85.8	9.3		
			925.2	81.7	86.3	7.6		
			857.2	116.6	79.9	10.9		
			916.3	98.9	85.4	9.2		
		Wet	876.1	126.9	81.7	11.8	75.4	10.8
			852.7	135.0	79.5	12.6		
			690.9	105.0	64.4	9.8		
			778.3	112.0	72.6	10.4		
			844.6	97.8	78.8	9.1		
	Acetone	Dry	1673	84.6	87.4	4.4	86.3	4.8
			1636	91.0	85.4	4.8		
			1628	92.9	85.0	4.9		
			1650	99.4	86.2	5.2		
			1676	89.5	87.5	4.7		
		Wet	1649	101.3	86.1	5.3	80.4	5.1
			1469	101.6	76.7	5.3		
			1535	112.7	80.2	5.9		
			1498	86.8	78.2	4.5		
			1551	90.5	81.0	4.7		
Solvent spike control	Methanol	n/a	1093	n/a				
			1062					
			1063					
	Acetone	n/a	1923					
			1903					
			1919					

n/a, not applicable.

3.7.6.3 Uptake and Extraction Efficiency Verification Test: Acetone

Based on the lessons learned from the additional scoping work with extraction efficiencies, the full uptake and extraction efficiency test was conducted again using acetone as the solvent, 20 mL as the extraction volume, and the 60 mL jar as the vessel. Furthermore, the DVB pads were extracted again for an additional 30 min in a second jar of solvent. The summary results for the acetone extraction are shown in Table 28 for the extraction efficiency and Table 29 for the uptake efficiency. The individual sample results are shown in Table 30 for the extraction efficiency and Table 31 for the uptake efficiency.

Table 28. Summary Extraction Efficiency Results: Acetone

Extraction Volume (mL)	Target Mass (ng)	1st Extraction			2nd Extraction		
		Average (%)	StDev (%)	RSD (%)	Average (%)	StDev (%)	RSD (%)
20	600	76.3	0.7	0.8	4.7	0.4	8.8
	4,000	86.5	1.3	1.5	4.8	0.5	9.4
	18,000	90.4	0.5	0.5	4.9	0.2	3.6

Table 29. Summary Uptake Extraction Results: Acetone

Extraction Volume (mL)	Sample Type	Target Mass (ng)	1st Extraction			2nd Extraction		
			Average (%)	StDev (%)	RSD (%)	Average (%)	StDev (%)	RSD (%)
20	DVB	600	84.7	10.6	12.6	4.1	0.5	13.1
		4,000	84.5	1.9	2.2	4.5	0.3	7.0
		18,000	82.7	4.4	5.3	6.7	2.3	34.9
	PTFE	600	99.5	0.9	0.9	n/a		
		4,000	99.8	0.3	0.3			
		18,000	>99.9	n/a	n/a			

n/a, not applicable.

Table 30. Extraction Efficiency Results: 20 mL Acetone Extraction

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average Efficiency (%)	
		1st Extraction	2nd Extraction	1st Extraction	2nd Extraction	1st Extraction	2nd Extraction
DVB extraction	600	464.6	32.7	75.7	5.3	76.3	4.7
		464.0	28.0	75.6	4.6		
		468.2	25.7	76.3	4.2		
		473.5	27.9	77.2	4.6		
		469.7	29.3	76.6	4.8		
	4,000	3,390	205	85.9	5.2	86.5	4.8
		3,502	204	88.8	5.2		
		3,376	202	85.6	5.1		
		3,371	168	85.5	4.3		
		3,423	174	86.8	4.4		
	18,000	16,321	900	90.4	5.0	90.4	4.9
		16,330	848	90.5	4.7		
		16,387	925	90.8	5.1		
		16,159	873	89.5	4.8		
		16,361	918	90.6	5.1		
Solvent spike control	600	610.4	n/a				
		615.7					
		614.4					
	4,000	3,967					
		3,922					
		3,944					
	18,000	18,213					
		18,040					
		17,906					

n/a, not applicable.

Table 31. Uptake Efficiency Results: 20 mL Acetone Extraction

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average (%)	
		1st Extraction	2nd Extraction	1st Extraction	2nd Extraction	1st Extraction	2nd Extraction
DVB extraction	600	520.5	26.4	92.0	4.7	84.7	4.1
		509.8	23.4	90.1	4.1		
		470.1	25.3	83.1	4.5		
		378.0	18.4	66.8	3.3		
		518.6	22.7	91.6	4.0		
	4,000	3,122	194.2	80.5	5.0	84.5	4.5
		3,316	181.5	85.5	4.7		
		3,277	163.3	84.5	4.2		
		3,256	162.4	83.9	4.2		
		3,426	169.9	88.3	4.4		
	18,000	16,245	904.1	87.4	4.9	82.7	6.7
		15,051	1,369	81.0	7.4		
		14,185	1,958	76.3	10.5		
		15,975	961.7	86.0	5.2		
		15,396	1,020	82.9	5.5		
PTFE sample	600	BQL	n/a	>99.9	n/a	99.5	n/a
		11.7		97.9			
		BQL		>99.9			
		BQL		99.7			
		BQL		>99.9			
	4,000	11.9		99.7		99.8	
		BQL		>99.9			
		BQL		99.9			
		BQL		>99.9			
		22.3		99.4			
	18,000	4.4		>99.9		>99.9	
		BQL		>99.9			
		BQL		>99.9			
		BQL		>99.9			
		BQL		>99.9			
PTFE control	600	561.8	n/a				
		573.2					
		562.8					
	4,000	3,860					
		3,896					
		3,883					
	18,000	18,215					
		19,452					
		18,078					

n/a, not applicable.
BQL, below quantification limit.

3.7.7 Uptake and Extraction Efficiency Discussion: 24 h Contact

When the acetonitrile efficiency results were examined, a difference was noted between the 10 and 20 mL extraction volumes. This was attributed to the solvent volume and not the vessel configuration. Therefore, 20 mL was used in each subsequent extraction, and a 60 mL jar was used for extractions to take advantage of the smaller waste profile.

During the initial uptake efficiency test with a 10 mL acetonitrile extraction, some of the samples may have been inadvertently mislabeled. This affected the 200 ng condition of the uptake efficiency testing. These samples are marked with an asterisk in Table 25.

A multivariate analysis was conducted to evaluate the effects of concentration and extraction time on efficiency values. The 20 mL acetonitrile extraction efficiency results were compared with respect to the various concentrations and extraction times. The *p* values for the analysis showed that extraction efficiency was strongly correlated with concentration but not with extraction time.

The efficiency testing with acetonitrile yielded results that were not as high as anticipated. Therefore, additional scoping tests were conducted to evaluate several potential parameters, including choice of solvent, dry versus prepared DVB pads, and single versus double extractions. In addition to these scoping tests, the potential for reaction products was examined. None were identified during testing with the Direct Analysis in Real Time (DART) instrument, a highly sensitive ionizer connected to a time-of-flight mass spectrometer.

The path forward was to use acetone as the extraction solvent. This was the same solvent used in the S&T V&V performed by Battelle and ECBC personnel.[3] The extraction volume was chosen to be 20 mL. The 60 mL jar was selected as the extraction vessel to reduce the waste stream. The comparison of single to double extractions indicated that it was not worth the additional costs and burdens associated with performing the second extraction. This decision was made during a teleconference between DUSA-TE, WDTC, ECBC, and Joint Project Manager for Protection (JPM P) personnel on 31 March 2014.

An additional discussion regarding multiple time-point efficiencies, including 48 h performance, is provided in Section 3.8.2.

3.8 Uptake and Extraction Efficiency Testing: Additional Time Points

3.8.1 Testing for 48 h

The Contaminated Human Remains Pouch (CHRP) program and other programs have a test requirement that is longer than 24 h. Additional verification testing was performed to address test periods of up to 48 h. It was an assumption that a system meeting the temperature-mapping verification requirements for 24 h would also be able to meet them for 48 h. Although a new profile map was not generated in support of this longer time duration, the temperature was logged during the 48 h trial. The only additional verification tests were uptake efficiency and extraction efficiency.

This testing followed the same procedures detailed in Section 3.7.4, with the following changes:

- The 48 h testing was limited to a single extraction time period (30 min) and a single extraction jar size (60 mL).
- The 48 h testing utilized 48 h of contact before the DVB pad was extracted.

- An additional series of extraction efficiencies was included in which the DVB pads were extracted after 1 min of contact. This was performed to better compare the extraction data to the contact time used during the S&T V&V conducted by Battelle and ECBC personnel.[3]

The test parameters that remained the same included the following:

- The same number of spike concentrations (three) was used.
- The same number of uptake efficiency replicates (five) was used for each spike concentration.
- The same number of uptake efficiency control samples (three) was used for each spike concentration.
- The same number of extraction efficiency replicates (five) was used for each spike concentration.
- The same number of extraction efficiency control samples (three) was used for each spike concentration.

The summary extraction efficiency results comparing 1 min versus 48 h contact prior to extraction are provided in Table 32. The 48 h uptake efficiency results are summarized in Table 33. The individual sample results for the 48 h extraction efficiency and uptake efficiency test are provided in Tables 34 and 35, respectively.

The efficacy reporting requirements included a completed run sheet and the tabulated data of the individual sample concentrations for the DVB extractions and the controls. The efficiency for each sample compared with the control average had to be reported, along with the average uptake efficiency, the standard deviation, and the relative standard deviation. It was required that the run sheet document the individual sample identification numbers, sample positions, spike times, solvent addition times, aliquot removal times, and observations.

The method acceptance limits for efficiency included values that were within 30% of the target control for each concentration tested.

Table 32. Summary Extraction Efficiency Results: Acetone, 1 min and 48 h Contact

Extraction Volume (mL)	Target Mass (ng)	1 min Contact			48 h Contact		
		Average (%)	StDev (%)	RSD (%)	Average (%)	StDev (%)	RSD (%)
20	600	95.2	1.2	1.2	71.7	1.4	1.9
	4,000	98.5	3.6	3.6	81.9	3.4	1.5
	18,000	96.2	3.9	4.1	80.5	1.5	1.8

Table 33. Summary Uptake Extraction Results: Acetone, 48 h Contact

Extraction Volume (mL)	Sample Type	Target Mass (ng)	48 h Contact		
			Average (%)	StDev (%)	RSD (%)
20	DVB	600	71.3	13.8	19.4
		4,000	72.5	10.5	14.5
		18,000	59.3	5.6	9.4
	PTFE	600	99.5	0.9	0.9
		4,000	>99.9	n/a	n/a
		18,000	>99.9	n/a	n/a

Table 34. Extraction Efficiency Results: 20 mL Acetone Extraction, 1 min and 48 h Contact

Sample Type	Mass Applied (ng)	Mass Recovered (ng)		Efficiency (%)		Average Efficiency (%)	
		1 min Contact	48 h Contact	1 min Contact	48 h Contact	1 min Contact	48 h Contact
DVB extraction	600	600.0	452.4	96.6	72.8	95.2	71.8
		584.4	450.8	94.1	72.6		
		596.3	432.5	96.0	69.6		
		592.3	442.0	95.4	71.2		
		583.6	450.4	94.0	72.5		
	4,000	4,092	3,404	104.6	87.0	98.5	81.9
		3,769	3,282	96.3	83.9		
		3,830	3,122	97.8	79.8		
		3,739	3,118	95.5	79.7		
		3,841	3,111	98.1	79.5		
	18,000	21,356	16,703	102.6	80.2	96.2	80.5
		19,941	16,305	95.8	78.3		
		20,108	16,765	96.6	80.5		
		19,313	17,120	92.7	82.2		
		19,405	16,967	93.2	81.5		
Solvent spike control	600	620.9	n/a				
		617.9					
		624.3					
	4,000	3,938					
		3,911					
		3,893					
	18,000	20,648					
		20,801					
		21,021					

n/a, not applicable.

Table 35. Uptake Efficiency Results: 20 mL Acetone Extraction, 48 h Contact

Sample Type	Mass Applied (ng)	Mass Recovered (ng)	Efficiency (%)	Average Efficiency (%)
DVB extraction	600	354.2	57.5	71.3
		541.2	87.8	
		459.6	74.6	
		492.2	79.9	
		348.9	56.6	
	4,000	2,804	74.5	72.5
		3,057	83.3	
		2,984	84.9	
		2,060	63.0	
		2,739	86.3	
	18,000	12,605	62.9	59.3
		12,356	65.2	
		10,320	58.4	
		8,392	50.6	
		9,910	59.2	
PTFE sample	600	13.4	97.8	99.5
		BQL	>99.9	
		BQL	>99.9	
		BQL	>99.9	
		BQL	>99.9	
	4,000	BQL	>99.9	>99.9
		BQL	>99.9	
		BQL	>99.9	
		BQL	>99.9	
		BQL	>99.9	
	18,000	BQL	>99.9	>99.9
		BQL	>99.9	
		BQL	>99.9	
		BQL	>99.9	
		BQL	>99.9	
PTFE control	600	617.8	n/a	
		610.7		
		620.0		
	4,000	3,676		
		3,748		
		3,861		
	18,000	19,981		
		20,099		
		20,008		

BQL, below quantification limit.
n/a, not applicable.

3.8.2 Uptake and Extraction Efficiency Discussion: Multiple Contact Time Points

In this experiment, extraction efficiency data were collected for pre-extraction contact periods of 1 min and 48 h at three VX target concentrations. These results were combined with those from the previous 24 h contact period to support a time-based analysis of the efficiencies. The results are shown graphically in Figure 27. A trend was noted that higher efficiencies

were achieved with shorter contact durations. A multivariate analysis indicated that the pre-extraction contact period length and the target concentration were factors that affected extraction efficiency.

It was not clear whether this dependence was due to greater binding between the analyte and the DVB pad, evaporation from the pad, some combination between them, or another unknown factor. Previous studies from decontamination programs have indicated that lower efficiencies are correlated with longer periods prior to extraction.[11]

Tables 32–35 fulfill the reporting requirements for the 48 h efficiency verification testing in support of the CHRP program. Although the extraction efficiency did not meet the original target of >90% efficiency, the 24 and 48 h test periods did meet the requirements in the EPA guidance for extraction efficiency performance with these DVB pads, which was 70–130%.

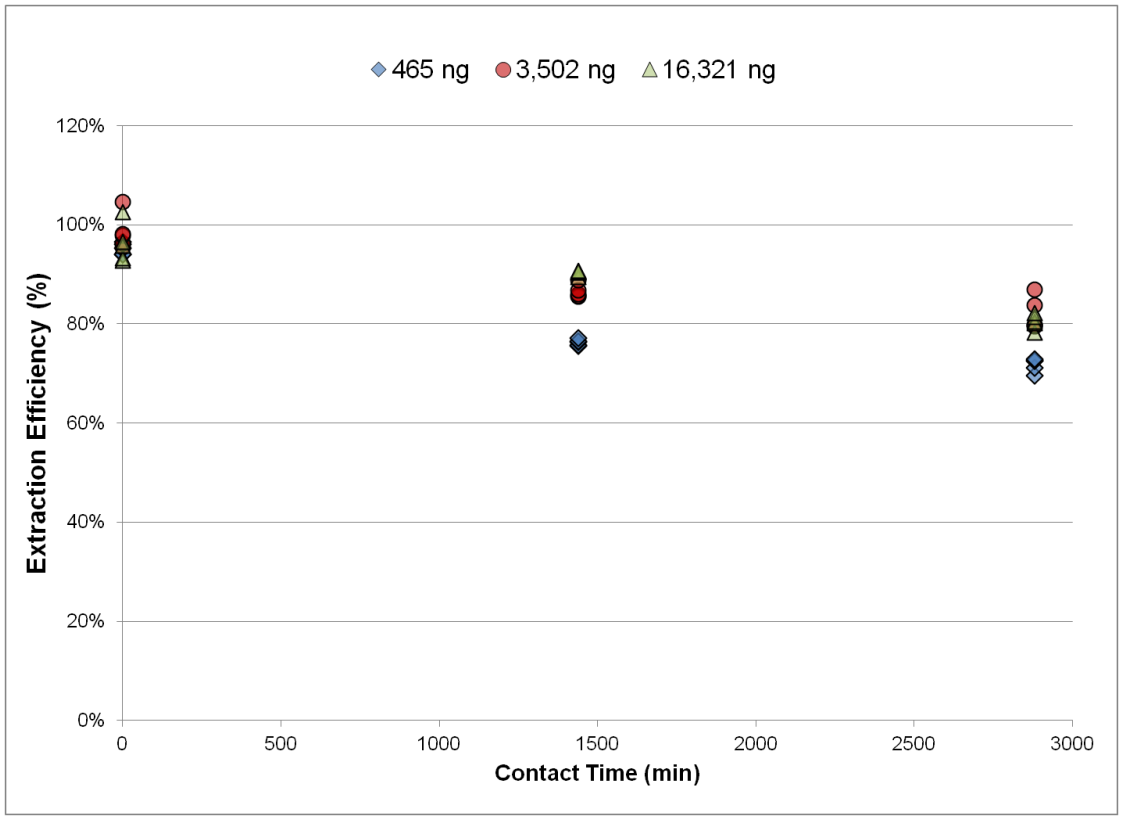


Figure 27. VX extraction efficiency results for various pre-extraction contact times and target VX masses.

3.9 Permeation Characterization Verification Test

3.9.1 Permeation Characterization Verification Test: Goals

The permeation characterization verification test had several goals. First, the background VX vapor concentration was measured with a butyl characterization sample. This value was an important component for establishing the practical reporting limit, based on sensitivity to background. Second, this test established the positive-control material for impermeable materials. Candidates included latex and neoprene. Third, this test identified the effectiveness of gasket sealing between the upper and lower swatch portions by measuring the differences between test samples with and without the gasket.

3.9.2 Permeation Characterization Verification Test: Experimental Procedures

The LVAP test cell is diagrammed in Figure 1. The contact test fixture consisted of a disposable polycarbonate Petri dish lined with a 2 in. diameter PTFE disk. A DVB sorbent pad was placed on the PTFE liner and covered with a 50 mm diameter swatch. The butyl swatches included an additional disk of aluminum foil between the DVB and the swatch to ensure permeation did not occur through the material, which isolated all measured response to the vapor background. The swatch was contaminated with six 1 µL drops placed in the middle of a 6 cm^2 area. The spiked swatch was photographed before the swatch was covered with a 28 mm diameter PTFE disk. The disk served as a protective layer for the 1 lb stainless steel weight. For samples that included it, the gasket was placed on the swatch before the weight was applied. This gasket had a 2 in. diameter and was the same as that used for the traditional AVLAG cell. The weight was then applied, and the sample was covered within an inverted 240 mL glass jar and placed within the incubator.

After a 24 h contact period had elapsed, the cell was removed from the incubator. The cell was photographed again once the weight had been removed. A fresh pair of disposable forceps was used to remove the DVB pad and place it in the solvent-extraction jar. Except for the weight, all other pieces were disposed of. The weight was rinsed with solvent over an appropriate waste container, allowed to dry, placed in a new jar, and stored in the incubator to await the next test. After extraction was complete, two aliquots of extract were removed. One aliquot was used for immediate analysis and the other was archived for future analysis (if needed). All extracts were stored at ≤4 °C and analyzed within 14 days.

3.9.3 Permeation Characterization Verification: Test Controls

Quantitative levels of VX permeated all latex and neoprene swatches tested. These swatches served as positive-control materials.

The negative control was an analyte-free matrix to which all reagents were added in the same volumes or proportions as those used in the sample processing. For each negative-control sample, the entire test process was completed using uncontaminated swatches. A negative-control sample was processed for each sample type.

A PTFE disk was spiked in the same manner as the swatch samples to verify that the spiking tool was operating properly, to confirm the proficiency of the operator, and to document the purity of the agent. After the PTFE disk was contaminated with the appropriate amount of agent, the spike disk was immediately extracted in 20 mL of acetone. An aliquot was removed for analysis at 30 min.

The experimental design was developed to distribute the samples randomly with negative-control samples distributed throughout the test matrix.

3.9.4 Permeation Characterization Verification: Test Results

Two permeation characterization tests were performed, Tests D and K. The second test was necessary because the foil was not applied within the butyl samples. The test results are summarized in Table 36 and presented graphically in Figure 28. Comprehensive results are shown in Table 37. An ANOVA was performed to compare the gasket versus no-gasket results for each material. The use of the gasket revealed a significant difference in the butyl results. However, the results for gasket versus no-gasket conditions were not statistically different for either the neoprene or latex. As noted in Section 6.3, a Wilcoxon method was used for the butyl results. Figure 28 is shown on a log scale to assist with visualization. The results were normally distributed.

The reporting requirements for the characterization verification test included a completed run sheet and the tabulated data of the individual sample concentrations for the DVB extractions, raw area integrations, and the controls. The measured responses for gasket versus no-gasket conditions were to be compared via appropriate statistical test, dependent on the distribution of the sets. Latex and neoprene sample masses were to be compared to establish the best positive-control material for the validation testing. The run sheet was to document the individual sample identification numbers, sample positions, spike times, aliquot removal times, and observations.

The minimum requirement for the positive-control samples was that the relative standard deviation between samples of the same type had to be less than 25%.

Tables 36 and 37 fulfill the reporting requirements for the permeation characterization verification test.

These results support several conclusions. First, the gasket was effective at reducing the potential for vapor cross-contamination into the DVB pad during 24 h contact periods with VX. Second, the use of the gasket did not change the overall permeation for positive-control materials. Third, both neoprene and latex met the standard deviation requirements for use as a positive control.

Table 36. Summary Characterization Results for Each Material Type: Gasket versus No Gasket

Material	Gasket Present	*n*	Average (ng)	StDev (ng)	RSD (%)	Measured Breakthrough (%)	*p* Value
Butyl	Yes	7	BQL	n/a	n/a	n/a	<0.001
	No	6	764	627	82.0	0.01	
Latex	Yes	10	4.57E+06	1.53E+05	3.3	76.1	0.900
	No	10	4.58E+06	1.26E+05	2.7	76.3	
Neoprene	Yes	10	9.66E+05	4.98E+04	5.2	16.1	0.445
	No	10	9.88E+05	7.47E+04	7.6	16.5	

BQL, below quantification limit.
n/a, not applicable.

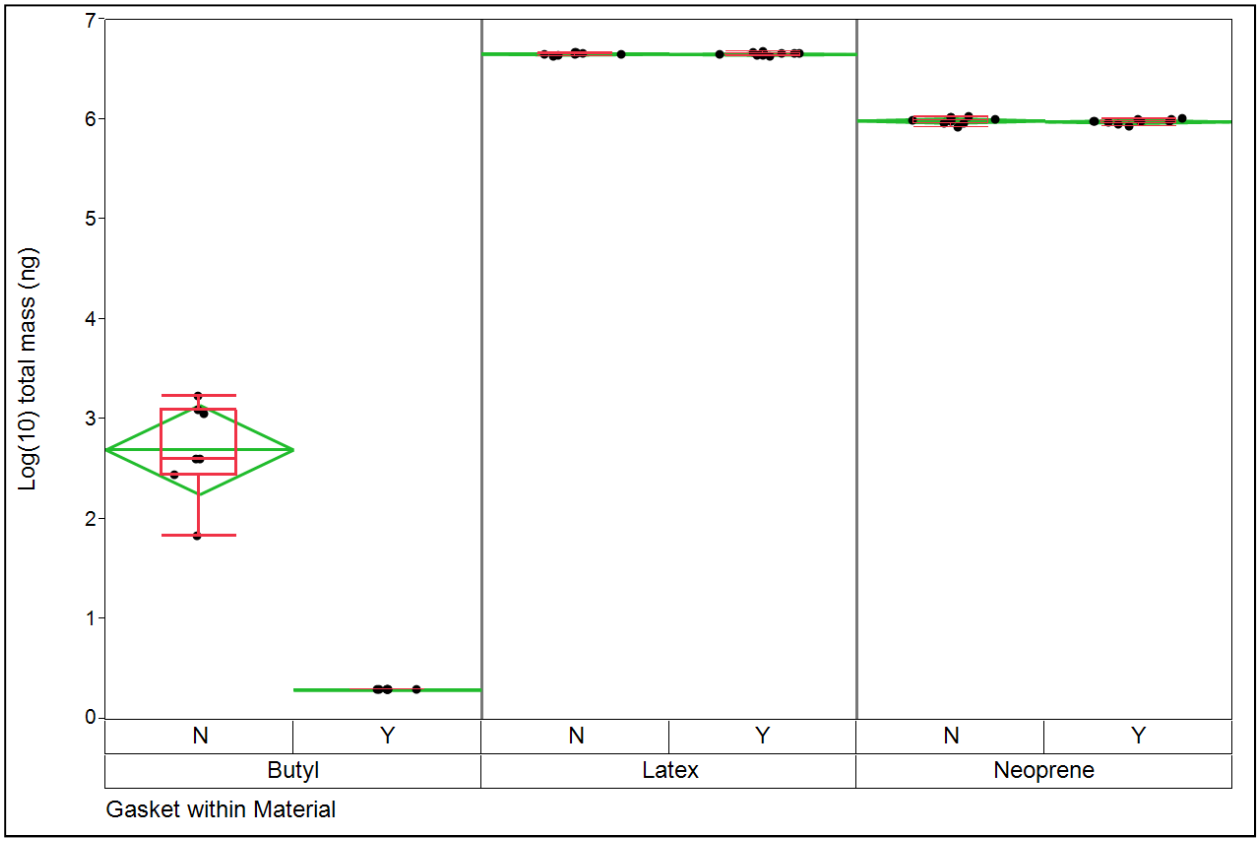


Figure 28. Comparison of gasket versus no-gasket results for each material.

Table 37. Comprehensive Permeation Characterization Results: Gasket versus No Gasket

Material	Gasket	Test ID	Position No.	Conc. (ng/mL)	Dilution	Area Count	Mass (ng)
Butyl	No	K	7	14.1	1	46,134	282.6
			15	63.8	1	41,471	1,276
			18	87.5	1	119,208	1,750
			24	20.5	1	66,856	409.0
			26	20.3	1	66,403	406.3
			27	3.5	1	11,531	69.6
			37	†	†	†	†
	Yes	K	1	BQL	1	228	BQL
			2	BQL	1	234	BQL
			16	BQL	1	227	BQL
			22	BQL	1	250	BQL
			23	BQL	1	223	BQL
			34	BQL	1	3,658	BQL
			36	BQL	1	2,798	BQL
Latex	No	D	2	2.34E+05	1000	120,142	4.68E+06
			4	2.35E+05	1000	120,514	4.70E+06
			8	2.30E+05	1000	118,144	4.60E+06
			11	2.19E+05	1000	113,047	4.38E+06
			13	2.30E+05	1000	117,938	4.59E+06
		K	4	2.32E+05	1000	120,261	4.64E+06
			8	2.22E+05	1000	115,186	4.43E+06
			21	2.28E+05	1000	118,181	4.56E+06
			32	2.33E+05	1000	120,494	4.65E+06
			38	2.38E+05	1000	129,555	4.76E+06
	Yes	D	3	2.37E+05	1000	121,602	4.75E+06
			23	2.25E+05	1000	115,808	4.50E+06
			24	2.13E+05	1000	109,946	4.25E+06
			34	2.16E+05	1000	111,527	4.32E+06
			39	2.30E+05	1000	118,355	4.61E+06
		K	6	2.29E+05	1000	118,696	4.58E+06
			10	2.29E+05	1000	118,660	4.58E+06
			12	2.35E+05	1000	121,850	4.71E+06
			17	2.30E+05	1000	119,208	4.60E+06
			20	2.42E+05	1000	125,257	4.85E+06
Neoprene	No	D	1	5.25E+04	250	108,683	1.05E+06
			16	4.99E+04	250	103,611	9.97E+05
			21	5.29E+04	250	109,460	1.06E+06
			26	4.63E+04	250	96,825	9.27E+05
			27	5.29E+04	250	109,489	1.06E+06
		K	3	4.21E+04	250	88,977	8.43E+05
			9	4.79E+04	250	100,388	9.58E+05
			11	4.65E+04	250	97,705	9.31E+05
			30	5.11E+04	250	106,751	1.02E+06
			39	4.97E+04	250	109,482	9.94E+05
	Yes	D	7	5.06E+04	250	105,028	1.01E+06
			10	5.20E+04	250	107,747	1.04E+06
			17	4.72E+04	250	98,406	9.43E+05
			20	5.08E+04	250	105,418	1.02E+06
			35	4.69E+04	250	97,849	9.37E+05
		K	13	4.53E+04	250	95,327	9.07E+05
			14	4.84E+04	250	101,504	9.69E+05
			29	5.17E+04	250	107,969	1.03E+06
			33	4.36E+04	250	91,870	8.72E+05
			35	4.79E+04	250	105,734	9.58E+05

† Outlier with attribution, sample lost.
BQL, below quantification limit.

4. VALIDATION TESTING

This section describes the test steps involved for validation testing. For each validation test, a coversheet was used to document pertinent test information along with the run sheet for that particular test.

4.1 Validation Test: Experimental Procedures

Testing commenced once the ACP01 swatches were removed from the preconditioning chamber and sealed in the temporary storage jar.

A diagram of the LVAP test cell is shown in Figure 1. The contact test fixture consisted of a disposable polycarbonate Petri dish lined with a 2 in. diameter PTFE disk. A DVB sorbent pad was placed on the PTFE liner and covered with a 50 mm diameter swatch. The butyl swatches included an additional disk of aluminum foil between the DVB and the swatch to ensure permeation did not occur through the material, which isolated all measured response to the vapor background. The swatch was contaminated with six 1 µL drops placed in the middle of a 6 cm^2 area. A photograph of the spiked swatch was taken prior to covering the swatch with a 28 mm diameter PTFE disk and the placement of the O-ring gasket. The disk served as a protective layer for the 1 lb stainless steel weight. The gasket had a 2 in. diameter and was the same as that used for the traditional AVLAG cell. The weight was then applied. For vapor control samples used during the 24 h validation testing, an additional 2 in. PTFE disk was placed on top of the stainless steel weight and followed by another DVB sorbent pad. Finally, the sample was covered by an inverted 240 mL glass jar and placed within the incubator.

After the timed contact period had elapsed, the cell was removed from the incubator. The cell was photographed again once the weight had been removed. A set of stainless steel forceps was used to remove the 28 mm PTFE disk and contaminated swatch. The forceps were periodically wiped or rinsed during testing. A fresh pair of disposable forceps was used to remove the DVB pad and to place it in the solvent-extraction jar. Except for the weight, all other pieces were disposed of. The weight was rinsed with solvent over an appropriate waste container, allowed to dry, placed in a new jar, and stored in the incubator to await the next test. After extraction, two aliquots of extract were removed. One aliquot was used for immediate analysis, and the other was archived for future analysis (if needed). All extracts were stored at ≤4 °C and analyzed within 14 days.

4.2 Validation Test: Controls

Quantitative levels of VX permeated through all latex and APC01 swatches tested. These swatches served as positive-control materials.

The negative control was an analyte-free matrix to which all reagents were added in the same volumes or proportions as those used in sample processing. For each negative-control sample, the entire test process was completed using uncontaminated swatches. A negative-control sample was processed for each sample type.

A limited number of additional samples were used to measure the vapor off-gassing that could cross-contaminate the DVB pad. For the 24 h validation testing, this was accomplished by putting a separate DVB and PTFE disk on top of the stainless steel weight. For the 48 h validation testing, this was accomplished with butyl swatches over aluminum foil, where permeation was prevented by the combination of materials. These DVB pads were assigned individual sample numbers and were extracted for 30 min in 20 mL of acetone, in accordance with normal DVB analysis procedures.

A PTFE disk spiked in the same manner as the swatch samples was used to verify that the spiking tool was operating properly, to confirm the proficiency of the operator, and to document the purity of the agent. After the PTFE disk was contaminated with the appropriate amount of agent, the spiked disk was immediately extracted in 20 mL of acetone. An aliquot for analysis was removed at 30 min.

The experimental design was developed to distribute the samples randomly with negative-control and vapor characterization samples distributed throughout the test matrix.

4.3 Validation Test: Results

Five validation tests were performed, and latex data from two verification tests, D and K, were also used to measure the variability of the test method. Tests E and F were 24 h contact tests, and Tests H and M were 48 h contact tests. The test results are summarized in Table 38.

Comprehensive results for latex at a 24 h contact time are shown in Table 39 and graphically presented in Figure 29. Comprehensive results for latex at a 48 h contact time are shown in Table 40 for Validation Test 1 and Table 41 for Validation Test 2. Results for latex as obtained during verification testing are presented in Section 3.9. Comprehensive results for APC01 at a 24 h contact time are shown in Table 42.

The validation test reporting requirements included a table reporting the test number, measured concentration, analytical dilution factor, and total permeated contaminant mass for each sample. A summary table was also to be provided to show the average permeation for each material type, the standard deviation, and the relative standard deviation. The average was to be the mean or geometric mean as appropriate, based on the normality of the data. Tables 38–42 fulfill the reporting requirements for the validation tests.

The temperature requirement was not met during the preconditioning portion of Test F. However, the absolute humidity requirement was met. Test F results for APC01 were included for completeness and to support discussion of the effects of environmental conditioning on permeation results. However, the APC01 test results were not included in the statistical summaries unless explicitly indicated. The preconditioning issue did not affect the latex results for Test F.

It is important to note that there was one APC01 sample that yielded permeation values equivalent to approximately 6 times the average value. There was no assignable cause or reason to remove this sample as an outlier. However, without this single sample, the relative standard deviation decreases from 85 to 17%, which may be more representative of the actual variation.

A total of three samples from the 48 h validation tests did not meet initial analytical quality control (QC) standards. These were reanalyzed on a separate calibration curve with a different level of sensitivity. This does not impact the analysis, but the samples have a different level of area counts than other samples from that test.

Three of the latex samples in Test M were out of thickness specification. These are indicated as outliers with assignable cause. The results from these samples are included in Table 41 for completeness but are not incorporated into the statistics.

Table 38. Summary Results for Validation Data

Material	Contact Time (h)	*n*	Average (µg)	StDev (µg)	RSD (%)	Measured Breakthrough (%)
Latex	24	65	4,798	387.8	8.1	86.0
	48	62	5,326	260.2	4.8	95.4
APC01	24	34	16.41	13.99	85.3	0.29

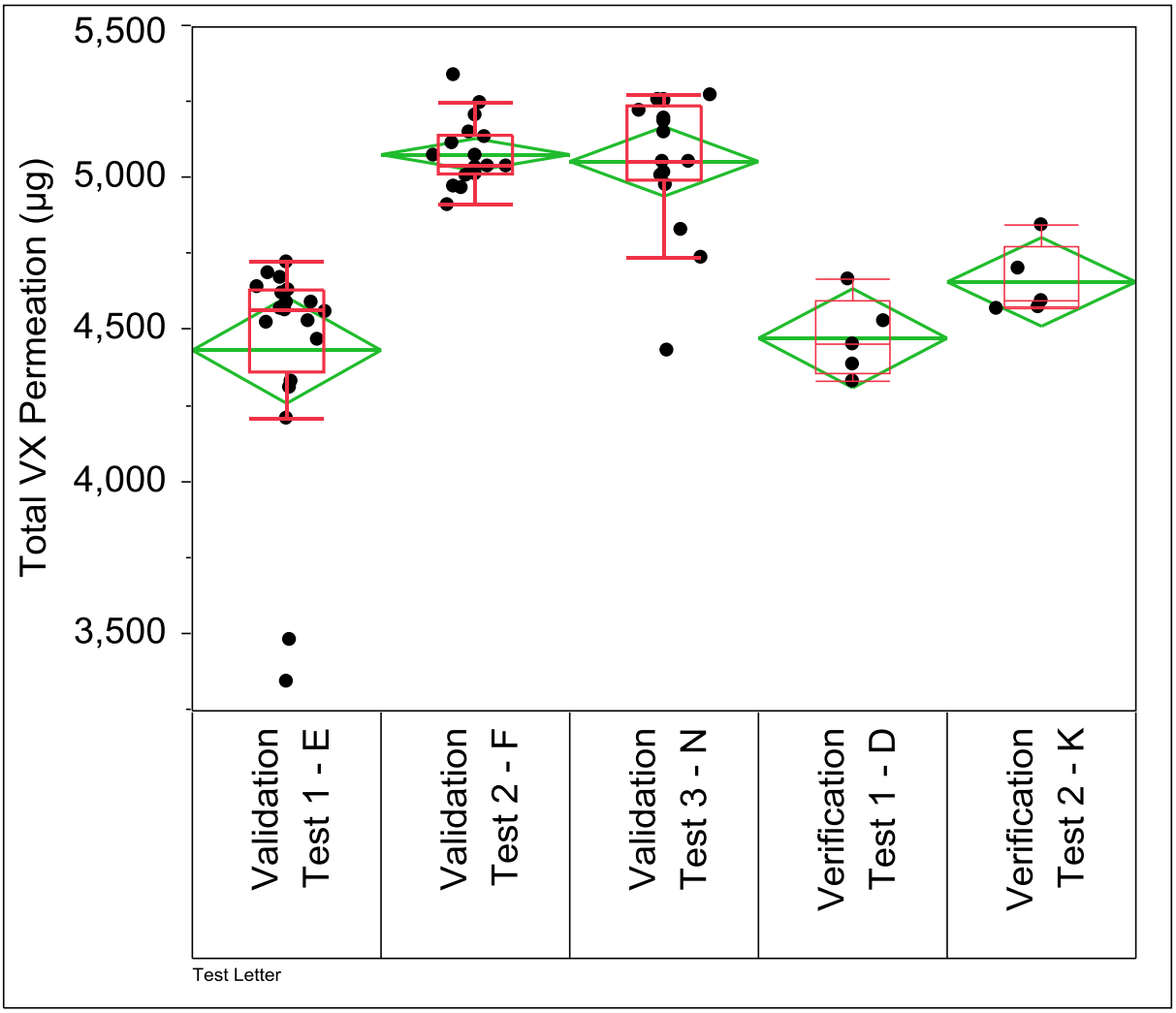


Figure 29. Plot of all data used for 24 h latex validation analysis.

Table 39. Comprehensive Latex Validation Test Results: 24 h

Material	Test ID	Position No.	Concentation (ng/mL)	Dilution	Area Count	Mass (µg)
Latex	E	1	2.31E+05	2,000	497,408	4,625
		3	2.28E+05	2,000	427,813	4,561
		7	2.29E+05	2,000	428,929	4,574
		8	2.10E+05	2,000	396,642	4,210
		9	1.67E+05	500	1,480,157	3,345*
		10	2.32E+05	2,000	434,414	4,636
		11	1.74E+05	2,000	382,979	3,485*
		12	2.27E+05	2,000	425,514	4,535
		14	2.35E+05	2,000	439,222	4,690
		15	2.17E+05	2,000	407,608	4,333
		16	2.16E+05	2,000	405,920	4,314
		17	2.36E+05	2,000	442,203	4,724
		20	2.28E+05	2,000	428,228	4,566
		21	2.32E+05	2,000	435,342	4,646
		26	2.31E+05	2,000	433,301	4,623
		29	2.26E+05	2,000	424,711	4,526
		32	2.30E+05	2,000	430,889	4,596
		36	2.30E+05	2,000	430,676	4,593
		37	2.24E+05	2,000	419,785	4,470
		38	2.34E+05	2,000	437,997	4,676
	F	1	2.62E+05	2,000	557,725	5,249
		2	2.56E+05	2,000	545,376	5,120
		3	2.49E+05	2,000	531,488	4,976
		8	2.46E+05	2,000	525,694	4,916
		9	2.58E+05	2,000	548,488	5,153
		11	2.51E+05	2,000	535,535	5,018
		13	2.52E+05	2,000	537,608	5,039
		14	2.49E+05	2,000	530,952	4,970
		16	2.52E+05	2,000	537,180	5,035
		17	2.51E+05	2,000	536,223	5,025
		23	2.52E+05	2,000	537,680	5,040
		24	2.60E+05	2,000	553,930	5,209
		26	2.51E+05	2,000	534,972	5,012
		31	2.54E+05	2,000	541,324	5,078
		33	2.54E+05	2,000	541,265	5,077
		36	2.67E+05	2,000	566,453	5,341
		37	2.52E+05	2,000	537,822	5,042
		40	2.57E+05	2,000	547,172	5,139
	N	2	2.22E+05	2,000	650,113	4,435
		3	2.59E+05	2,000	749,643	5,188
		6	2.37E+05	2,000	690,654	4,739
		11	2.53E+05	2,000	732,348	5,056
		13	2.51E+05	2,000	727,771	5,021
		14	2.42E+05	2,000	703,267	4,834
		18	2.51E+05	2,000	726,710	5,013
		19	†	†	†	†
		21	2.60E+05	2,000	751,050	5,199
		23	2.51E+05	2,000	727,978	5,022
		27	2.63E+05	2,000	758,111	5,254
		28	2.49E+05	2,000	722,739	4,982
		29	2.53E+05	2,000	732,761	5,059
		32	2.63E+05	2,000	759,015	5,261
		33	2.58E+05	2,000	745,409	5,156
		34	2.61E+05	2,000	754,350	5,225
		36	2.64E+05	2,000	760,717	5,274
		38	2.63E+05	2,000	758,876	5,260

*Sample considered a statistical outlier; cf. Section 6.4.3.
†Sample outlier with assignable cause: sample lost.

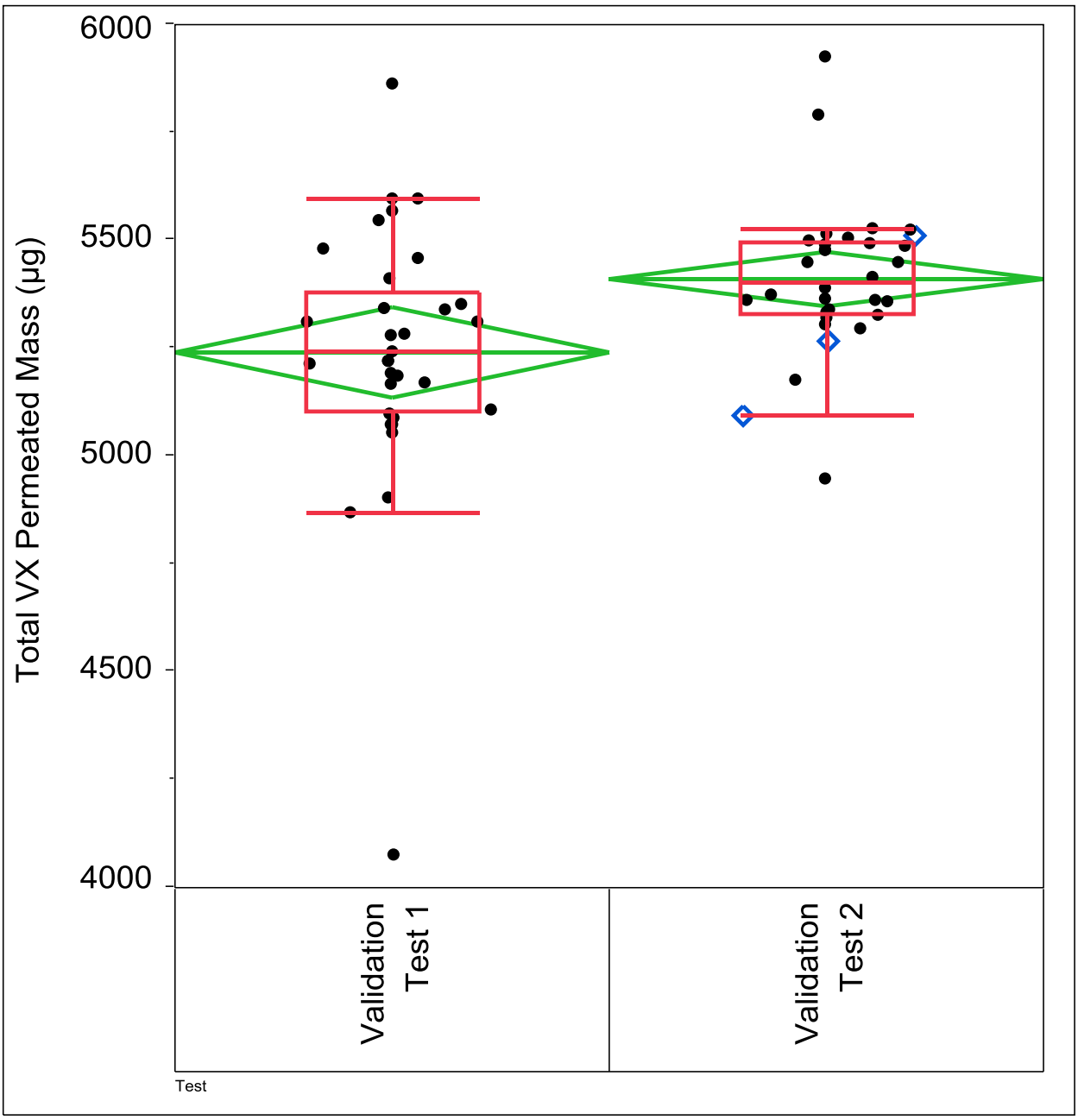


Figure 30. Plot of 48 h latex validation data. Blue diamonds indicate outlier samples that were outside the allowed thickness requirements.

Table 40. Comprehensive Latex Validation Test 1 Results: 48 h

Material	Test ID	Position No.	Concentration (ng/mL)	Dilution	Area Count	Mass (µg)
Latex	H	1	2.59E+05	2,000	912,872	5,310
		2	2.53E+05	2,000	872,748	5,052
		3	2.61E+05	2,000	898,892	5,220
		4	2.91E+05	2,000	996,937	5,861
		6	2.54E+05	2,000	878,371	5,088
		7	2.59E+05	2,000	902,275	5,242
		8	2.00E+05	2,000	716,742	4,074
		10	2.49E+05	2,000	876,024	5,073
		12	2.59E+05	2,000	907,844	5,278
		13	2.56E+05	2,000	881,222	5,107
		14	2.56E+05	2,000	890,528	5,166
		15	2.60E+05	2,000	907,988	5,279
		16	2.43E+05	2,000	843,771	4,868
		17	2.68E+05	2,000	912,738	5,310
		18	2.53E+05	2,000	879,834	5,098
		19	2.43E+05	2,000	875,660	5,071
		20	2.74E+05	2,000	96,689	5,479
		21	2.49E+05	2,000	893,222	5,184
		22	2.41E+05	2,000	849,371	4,903
		23	2.51E+05	2,000	890,916	5,169
		24	2.53E+05	2,000	894,259	5,190
		25	2.49E+05	2,000	897,567	5,212
		27	2.61E+05	2,000	928,293	5,410
		29	2.56E+05	2,000	918,967	5,350
		30	2.60E+05	2,000	908,448	5,282
		31	2.51E+05	2,000	898,621	5,218
		32	2.54E+05	2,000	917,943	5,343
		34	2.56E+05	2,000	952,196	5,566
		36	2.52E+05	2,000	948,993	5,545
		37	2.59E+05	2,000	956,635	5,595
		38	2.46E+05	2,000	916,891	5,337
		39	2.50E+05	2,000	935,383	5,457
		40	2.54E+05	2,000	956,705	5,596

Table 41. Comprehensive Latex Validation Test 2 Results: 48 h

Material	Test ID	Position No.	Concentration (ng/mL)	Dilution	Area Count	Mass (µg)
Latex	M	1	2.74E+05	2000	938,385	5,476
		2	2.65E+05	2000	910,762	5,296
		3	2.59E+05	2000	891,976	5,175
		4	2.66E+05	2000	915,521	5,327
		5	2.96E+05	2000	1,006,534	5,924
		6	2.71E+05	2000	929,082	5,415
		7	2.72E+05	2000	934,351	5,449
		8	2.69E+05	2000	922,523	5,372
		9	2.67E+05	2000	917,431	5,339
		11	2.72E+05	2000	934,129	5,448
		12	2.76E+05	2000	944,172	5,513
		13	2.76E+05	2000	945,790	5,524
		15	2.47E+05	2000	856,532	4,948
		17	2.68E+05	2000	920,458	5,359
		18	2.68E+05	2000	921,275	5,364
		20	2.74E+05	2000	938,356	5,475
		21	2.65E+05	2000	911,960	5,304
		22	2.75E+05	2000	940,884	5,492
		23	2.74E+05	2000	940,118	5,487
		24	2.74E+05	2000	940,296	5,488
		27	2.69E+05	2000	95,282	5,390
		28	2.66E+05	2000	914,412	5,320
		30	2.75E+05	2000	941,730	5,497
		31	2.75E+05	2000	942,815	5,505
		32	2.67E+05	2000	916,487	5,333
		33	2.76E+05	2000	946,191	5,527
		34	2.89E+05	2000	986,128	5,789
		35	2.68E+05	2000	920,373	5,358
		36	2.63E+05	2000	906,199	5,267†
		37	2.55E+05	2000	879,415	5,094†
		39	2.68E+05	2000	94,844	5,362
		40	2.76E+05	2000	943,909	5,512†

†Outliers with attributable cause: sample did not meet QC for thickness; cf. Section 5.1.3.

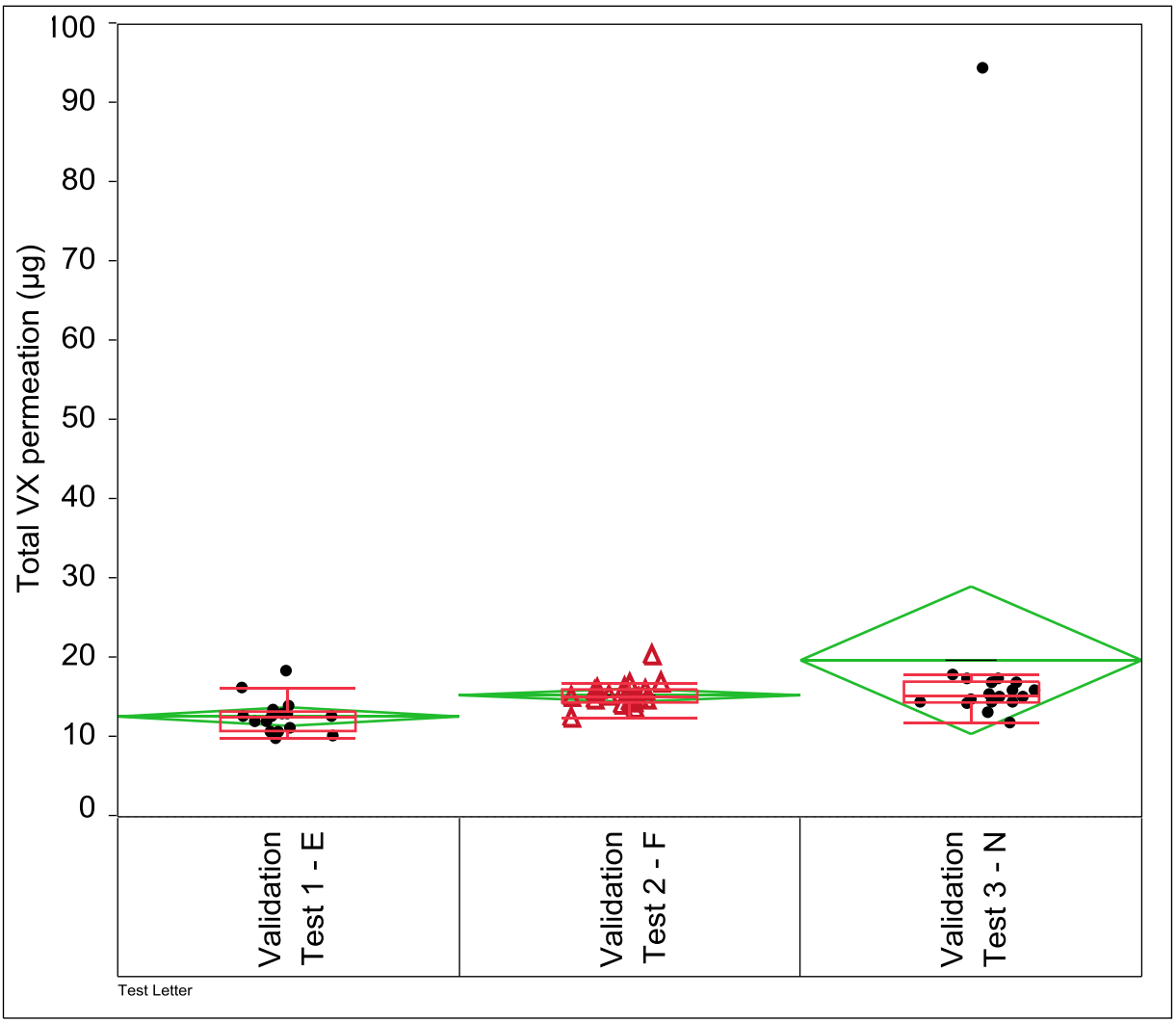


Figure 31. Plot of 24 h APC01 validation data. Test F did not meet the preconditioning temperature requirement, but met the absolute humidity requirement. Test N contained an extreme data point.

Table 42. Comprehensive APC01 Validation Test Results: 24 h

Material	Test ID	Position No.	Concentration (ng/mL)	Dilution	Area Count	Mass (µg)
APC01	E	2	649.8	6	407,497	13.00
		5	599.5	6	377,600	11.99
		6	490.5	6	311,959	9.81
		13	673.0	6	421,244	13.46
		18	917.2	6	562,430	18.34
		19	628.8	6	395,039	12.58
		22	629.3	6	395,371	12.59
		24	815.1	6	504,109	16.30
		25	599.7	6	377,709	11.99
		27	695.1	6	434,245	13.90
		28	510.4	6	324,050	10.21
		31	557.2	6	352,247	11.14
		34	533.2	6	337,819	10.66
		35	648.9	6	406,984	12.98
		39	535.9	6	339,423	10.72
		40	632.7	6	397,383	12.65
	F	4	805.5	6	569,228	16.11†
		6	836.6	6	588,835	16.73†
		7	741.0	6	528,014	14.82†
		10	1,018.5	6	699,927	20.37†
		12	743.8	6	529,843	14.88†
		15	757.5	6	538,647	15.15†
		18	620.9	6	449,296	12.42†
		20	721.1	6	515,183	14.42†
		21	678.3	6	487,274	13.57†
		22	785.8	6	556,704	15.72†
		27	809.8	6	571,960	16.20†
		28	759.1	6	539,680	15.18†
		29	733.9	6	523,425	14.68†
		30	843.4	6	593,076	16.87†
		32	719.8	6	514,329	14.40†
		34	708.9	6	507,257	14.18†
		35	798.1	6	564,526	15.96†
		39	722.5	6	516,085	14.45†
	N	1	754.1	6	728,648	15.08
		4	773.4	6	745,389	15.47
		5	586.6	6	579,058	11.73
		7	797.2	6	766,024	15.94
		8	840.4	6	803,027	16.81
		10	710.8	6	690,634	14.22
		12	738.9	6	715,379	14.78
		16	723.4	6	701,730	14.47
		20	652.3	6	638,618	13.05
		22	718.5	6	697,420	14.37
		24	870.1	6	828,193	17.40
		25	797.0	6	765,791	15.94
		26	842.8	6	805,063	16.86
		31	757.5	6	731,641	15.15
		35	871.8	6	829,610	17.44
		37	894.4	6	848,561	17.89
		39	723.0	6	701,410	14.46
		40	4,725.2	50	413,392	94.5*

†Outliers with attributable cause: samples did not meet preconditioning QC; cf. Section 6.4.3.
*Sample considered a statistical outlier; cf. Section 6.4.3.

4.4 Analytical Calibration and Controls for Validation Testing

The analytical instrument was an LC-MSMS. The instrument was calibrated with a minimum of five standards ranging from 0.52 to 520 ng/mL. A CCV was included within the range of the calibration curve. A CCV sample was analyzed at least once for every 10 samples. Based on analytical work performed during the verification portion of testing, the calibration curve for acetone was best described by a linear fit with $1/x$ weighting due to the heteroscedastic variability noted in the verification analysis.

During the verification analytical process described in Section 3.4, the lowest concentration calibration curve standard (0.118 ng/mL) was higher than the target for five of the seven replicates. Some of the results were outside the target range of ±20%. This was attributed to carryover between analyses. Therefore, the lowest calibration standard was increased to 0.52 ng/mL, and the dynamic range was adjusted to a maximum of 520 ng/mL. The smaller dynamic range helped to focus the instrument on the concentration of the samples being analyzed. This adjustment was noted within the verification report;[12] however, it is a deviation from the test plan.

Test samples submitted for analysis were diluted volumetrically to be within the calibration curve range. Combinations of class A glassware, class A pipettes, class A volumetric flasks, and gas-tight syringes were used in these dilutions.

Individual calibration curve results are plotted in Figure 32 and presented in Table 43. Individual CCV results are plotted in Figure 33 and presented in Table 44.

The reporting requirement was a table of the prepared standards that included raw integrated areas, calculated concentrations, and percent recoveries. Tables 43 and 44 fulfill the reporting requirements for the analytical results. All calibration curve and CCV data points met the required standards.

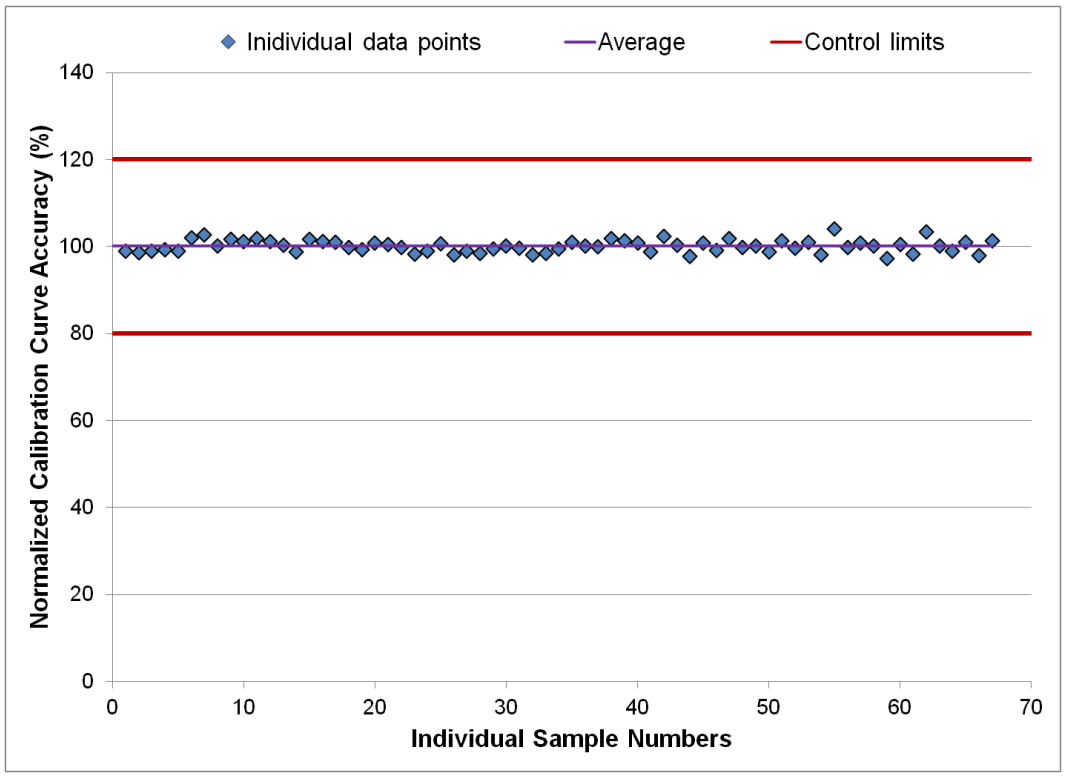


Figure 32. Individual accuracy results for calibration curve standards used during validation testing.

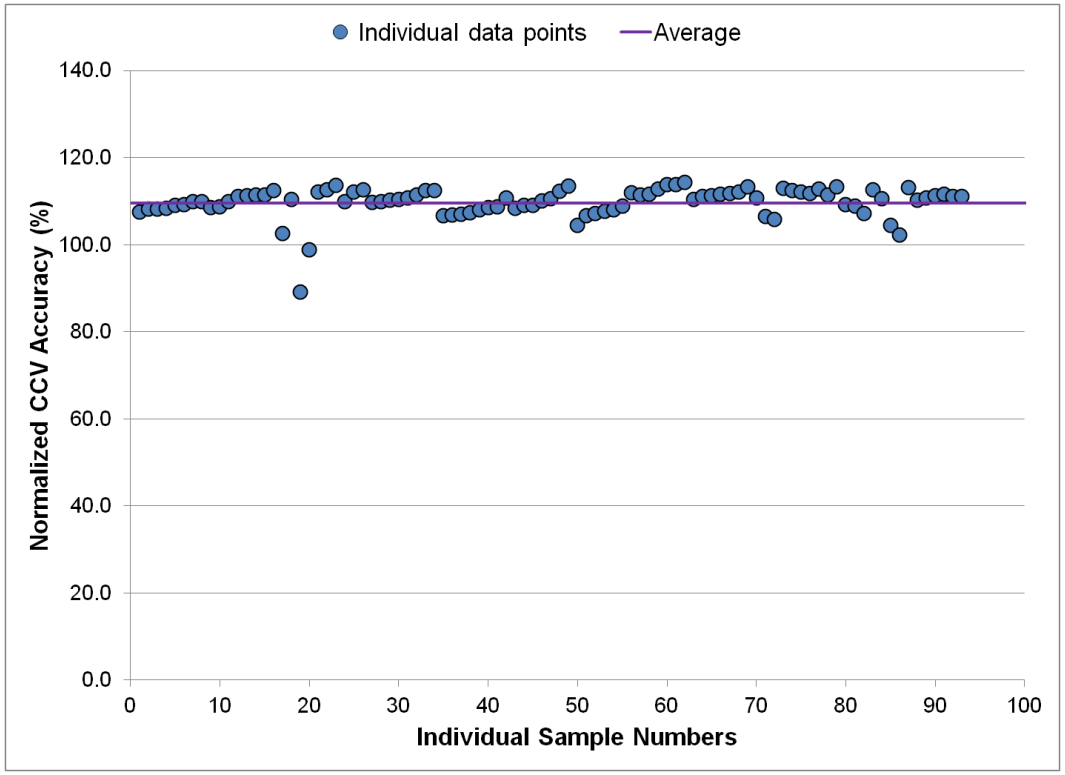


Figure 33. Individual accuracy results for CCV standards used during validation testing.

Table 43. Calibration Curve Results for Each Validation Test Sample Analytical Analysis

Target (ng/mL)	Test ID	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Target (ng/mL)	Test ID	Raw Response	Final Conc. (ng/mL)	Accuracy (%)
0.5	N	3,274	0.51	98.0	10.4	H	8,188	10.43	100.3
0.5	N	4,060	0.51	98.3	10.4	H	79,211	10.44	100.4
0.5	E	4,414	0.51	98.6	10.4	E	54,573	10.49	100.9
0.5	H	491	0.51	98.7	52.0	N	228,429	50.58	97.3
0.5	E	2,878	0.51	98.9	52.0	N	312,333	50.88	97.8
0.5	M	5,075	0.51	99.0	52.0	F	230,522	51.02	98.1
0.5	F	2,917	0.51	99.0	52.0	E	243,365	51.13	98.3
0.5	H	4,780	0.52	99.3	52.0	M	374,325	51.17	98.4
1.0	N	2,741	1.04	99.7	52.0	H	375,915	51.45	98.9
1.0	E	6,497	1.04	100.2	52.0	E	199,916	51.45	98.9
1.0	H	8,821	1.05	101.1	52.0	H	38,940	51.57	99.2
1.0	F	5,451	1.06	101.7	52.0	N	114,592	51.80	99.6
1.0	M	9,191	1.06	101.9	52.0	E	262,999	52.32	100.6
1.0	E	5,574	1.06	102.0	104.0	H	73,824	101.69	97.8
1.0	H	922	1.06	102.4	104.0	F	443,384	102.01	98.1
1.0	E	6,596	1.07	102.8	104.0	H	719,972	102.34	98.4
1.0	N	7,632	1.07	103.3	104.0	N	218,316	102.66	98.7
1.0	N	5,932	1.08	104.0	104.0	N	606,849	102.85	98.9
5.2	E	27,355	5.13	98.7	104.0	E	472,376	103.38	99.4
5.2	N	25,028	5.20	100.1	104.0	M	727,708	103.49	99.5
5.2	E	22,826	5.21	100.2	104.0	E	508,730	103.62	99.6
5.2	H	4,177	5.24	100.8	104.0	E	392,550	104.09	100.1
5.2	N	34,006	5.25	100.9	104.0	N	454,959	104.78	100.7
5.2	M	40,746	5.25	101.0	520.0	E	2,101,639	520.02	100.0
5.2	E	26,203	5.26	101.1	520.0	E	1,579,107	520.90	100.2
5.2	H	40,487	5.26	101.2	520.0	N	1,599,202	522.06	100.4
5.2	N	12,387	5.27	101.4	520.0	M	2,533,397	524.13	100.8
5.2	F	25,116	5.28	101.6	520.0	N	757,205	524.72	100.9
10.4	E	42,740	10.32	99.2	520.0	E	1,604,604	525.31	101.0
10.4	N	48,820	10.37	99.7	520.0	H	2,545,858	526.45	101.2
10.4	E	51,162	10.37	99.7	520.0	N	2,114,422	527.25	101.4
10.4	M	79,072	10.38	99.8	520.0	F	1,558,990	529.22	101.8
10.4	N	66,428	10.41	100.1	520.0	H	256,344	529.33	101.8
10.4	N	24,022	10.41	100.1					

Table 44. CCV Sample Results for Each Analytical Analysis: 10.1 ng/mL

Target (ng/mL)	Test ID	Raw Response	Final Conc. (ng/mL)	Accuracy (%)	Target (ng/mL)	Test ID	Raw Response	Final Conc. (ng/mL)	Accuracy (%)
10.1	E	44,825	10.85	107.4	10.1	H	83,526	11.02	109.1
	E	45,108	10.93	108.2		H	84,238	11.12	110.1
	E	45,115	10.93	108.2		H	84,667	11.17	110.6
	E	45,150	10.94	108.3		H	85,876	11.34	112.3
	E	45,422	11.01	109.0		H	86,757	11.46	113.4
	E	45,541	11.04	109.3		M	85,625	11.26	111.5
	E	45,773	11.10	109.9		M	85,748	11.27	111.6
	E	45,797	11.10	109.9		M	86,658	11.40	112.8
	E	53,935	10.36	102.6		M	87,392	11.50	113.8
	E	57,931	11.15	110.4		M	87,404	11.50	113.8
	E	55,737	11.32	112.0		M	87,764	11.55	114.3
	E	55,991	11.37	112.5		N	26,355	11.45	113.3
	E	56,548	11.48	113.7		N	25,763	11.18	110.7
	F	51,945	11.09	109.8		N	50,558	10.75	106.4
	F	51,986	11.10	109.9		N	50,261	10.68	105.8
	F	52,169	11.14	110.3		N	72,618	11.40	112.9
	F	52,227	11.15	110.4		N	72,373	11.36	112.5
	F	52,371	11.18	110.7		N	72,142	11.32	112.1
	F	52,721	11.26	111.4		N	71,921	11.29	111.8
	F	53,155	11.35	112.4		N	72,511	11.38	112.7
	F	53,165	11.35	112.4		N	71,676	11.25	111.4
	H	82,894	10.94	108.3		N	72,916	11.45	113.3
	H	83,482	11.02	109.1					

Table 45. CCV Sample Results for Each Analytical Analysis: 101 ng/mL

Target (ng/mL)	Test ID	Raw Response	Final Conc. (ng/mL)	Accuracy (%)		Target (ng/mL)	Test ID	Raw Response	Final Conc. (ng/mL)	Accuracy (%)
101	E	412,043	109.57	108.5		101	H	761,481	108.76	107.7
	E	412,780	109.78	108.7			H	763,778	109.12	108.0
	E	417,121	111.00	109.9			H	769,219	109.96	108.9
	E	421,425	112.22	111.1			H	788,706	113.01	111.9
	E	422,090	112.41	111.3			H	79,563	110.34	109.2
	E	422,459	112.51	111.4			H	79,365	110.04	109.0
	E	422,554	112.54	111.4			H	78,225	108.31	107.2
	E	426,399	113.63	112.5			M	779,656	111.56	110.5
	E	444,911	90.08	89.2			M	783,407	112.15	111.0
	E	491,023	99.85	98.9			M	784,431	112.31	111.2
	E	504,152	111.01	109.9			M	786,986	112.71	111.6
	E	513,277	113.22	112.1			M	787,701	112.82	111.7
	E	515,207	113.68	112.6			M	789,844	113.15	112.0
	F	466,431	107.78	106.7			N	239,746	113.71	112.6
	F	467,211	107.98	106.9			N	235,897	111.71	110.6
	F	467,607	108.08	107.0			N	457,968	105.53	104.5
	F	468,689	108.35	107.3			N	448,841	103.26	102.2
	F	471,933	109.17	108.1			N	668,456	114.29	113.2
	F	473,876	109.66	108.6			N	652,632	111.33	110.2
	F	474,261	109.76	108.7			N	655,725	111.91	110.8
	F	482,268	111.78	110.7			N	658,234	112.38	111.3
	H	740,920	105.57	104.5			N	660,421	112.79	111.7
	H	754,272	107.64	106.6			N	657,678	112.28	111.2
	H	758,560	108.31	107.2			N	657,344	112.21	111.1

5. QUALITY MANAGEMENT

5.1 Chain of Custody

The objective of the chain of custody was to ensure that test articles were traceable throughout all phases of testing. Guidance for sample receipt and chain of custody procedures were obtained from the ISO/IEC 17025:2005 standard[13] as well as the current version of Permeation and Analytical Solutions Branch (PASB) Internal Operating Procedure number 014.

5.1.1 Test Item Security

The location where the samples were received, processed, and tested was a secure facility with limited access at all times.

5.1.2 Initial Receipt Inspections of Test Items

Materials processed and cut as swatches were inspected for imperfections and damage. No defects were noted in the materials.

5.1.3 Swatch Processing

After the swatches were cut, the thickness of each sample was measured using a thickness gauge at three random locations on the sample. During measurement, nothing impeded the contact point between the gauge and the sample area, which would have produced a false measurement. Each measurement was automatically transferred to a Microsoft Excel spread sheet via computer connection to the thickness gauge. The thickness measurements are summarized in Table 46. Here, the butyl and neoprene results are for verification tests only, and the latex results are for verification and validation tests.

There are three items of note regarding the thickness measurements. First, the average latex thickness was greater than anticipated from the product information. However, the standard deviation from the mean was still within ±0.05 mm. A histogram of all latex thickness results is shown in Figure 34. Here, swatches with thicknesses between 0.26 and 0.36 mm were acceptable. Second, three swatches from the 48 h Validation Test 2, Test M, did not meet the thickness tolerance requirement. These were included in the thickness histogram of Figure 34, but not in the summary statistics of Table 46. Furthermore, the permeation results from these samples were denoted as outliers with attribution in Section 4.3. Third, the operators did not measure the swatch thicknesses for the 24 h Validation Test 1, Test E. However, triplicate measurements were obtained from 10 locations of the remainder of the bulk sheet material from where the swatches for Test E were obtained. These were within the average for the other swatches and were included in the summary statistics of Table 46. This oversight was not expected to impact the testing because both the thickness and permeation results were within the expected measurement ranges.

Table 46 fulfills the reporting requirements for the swatch thickness measurements.

Table 46. Summary of Swatch Thickness Measurements

Material	*n*	Average (mm)	StDev (mm)	RSD (%)	Range (mm)
Butyl	18	0.2352	0.0063	2.71	0.2244–0.2455
Latex	141	0.3105	0.0128	4.12	0.2752–0.3556
Neoprene	24	0.5089	0.0031	0.60	0.5080–0.5207

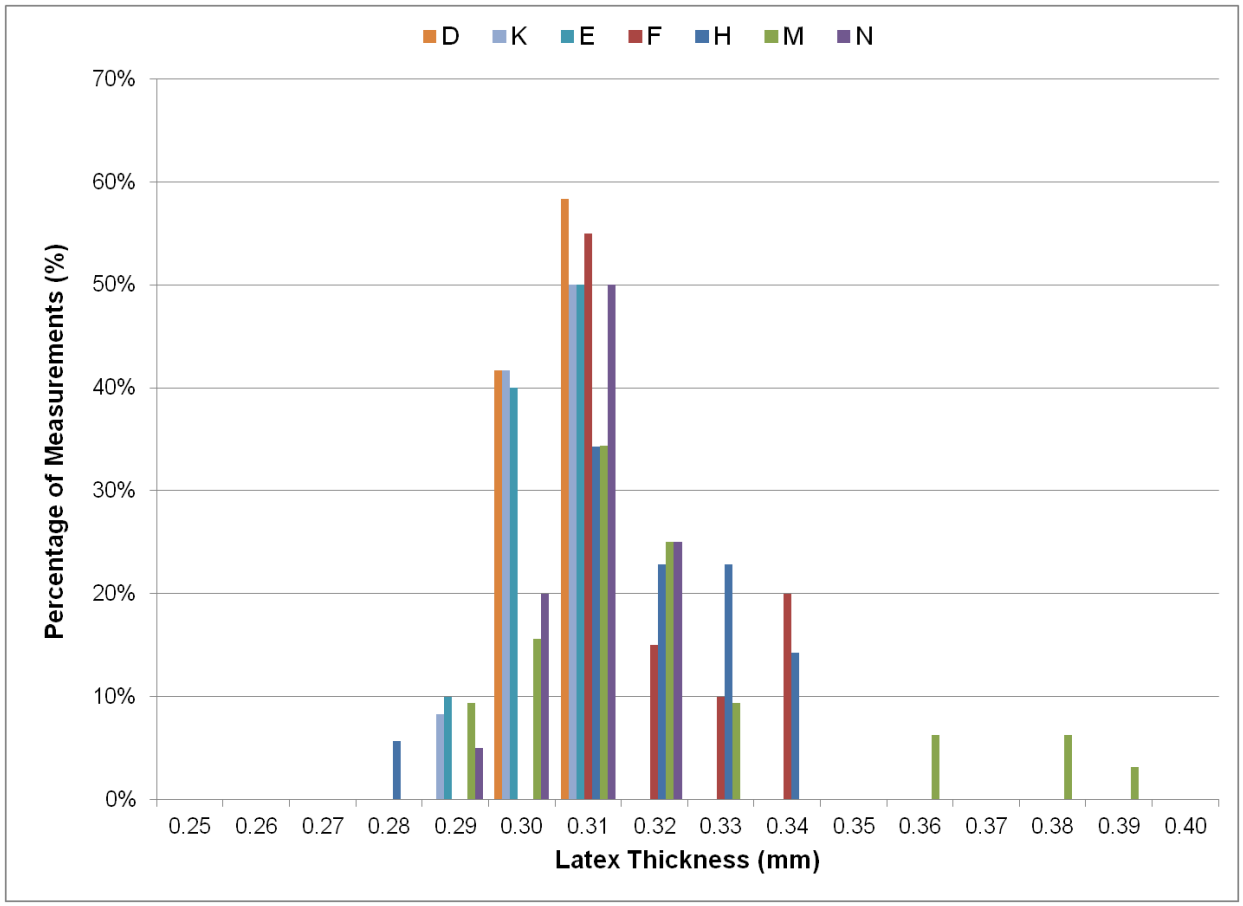


Figure 34. Latex thickness measurements.

5.2 Chemical Agent Quality

VX was the contaminant used for this test. The minimum purity requirement was 90%. Lot VX-U-1223-CTF-N was used, which had a purity >90%; however, this material was not a CASARM. A copy of the certification of analysis (CoA) is included in Appendix B.

During each neat agent test, at least two spiked samples on PTFE were performed to confirm agent purity, dosing tool function, and operator proficiency. This was accomplished by spiking six 1 µL droplets on PTFE, which was followed by extraction and analysis with LC-MSMS. This verification was performed at the beginning and end of each neat VX test. The results are provided in Table 47.

The CoA and Table 47 fulfill the reporting requirements for the VX purity. The purity requirement was met for the VX agent used during each test.

Table 47. VX Neat Agent Purity Results

Test Type	Test ID	Vial No.	Comment	Mass Recovered (µg)	Purity (%)
Verification	C	13	Operator no. 1 proficiency	5,580	93.0
				5,827	97.1
				5,801	96.7
				5,830	97.2
				5,815	96.9
				5,938	99.0
				5,734	95.6
				5,554	92.6
			Operator no. 2 proficiency	5,637	93.9
				5,759	96.0
				5,688	94.8
				5,691	94.9
				5,728	95.5
				5,803	96.7
				5,774	96.2
				5,681	94.7
	D	13	Start of test	5,482	91.4
			End of test	5,504	91.7
	K	13	Start of test	5,451	90.8
			End of test	5,476	91.3
Validation	E	14	Start of test	5,647	94.1
			End of test	5,687	94.8
	F	15	Start of test	5,997	99.9
			End of test	6,095	101.6
	H	15	Start of test	6,194	103.2
			End of test	6,359	106.0
	M	18	Start of test	6,168	102.8
			End of test	5,996	99.9
	N	17	Start of test	6,215	103.6
			End of test	6,231	103.8

5.3 Analytical Sample Storage

Analytical extract samples were stored at ≤4 °C. The purpose of this requirement was to preserve the extraction samples and protect them from degradation. This was achieved by documenting the maximum allowed storage temperature and duration and accepting temperatures and durations less than those. The maximum temperature and storage duration was in compliance with EPA SW-846, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods for Volatile Organic Compounds*.[5]

5.4 Quality Controls

Quality controls were implemented for each test.

5.4.1 Negative Controls

Negative-control samples were analyte-free matrices to which all reagents were added in the same volumes or proportions as those used in sample processing. The negative-control samples were carried through the complete sample preparation and analytical procedure. A negative control was used to document contamination resulting from the entire test process. Individual negative-control results are presented in Table 48 for the verification tests and Table 49 for the validation tests.

Table 48. Individual Negative-Control Sample Results: Verification

Test Type	Test ID	Position No.	Material	Sample No.	Result (ng)
Verification	A	16	DVB–30 min	6495	19.1
		16	PTFE–30 min	6511	6.0
		16	DVB–60 min	6560	15.1
		16	PTFE–60 min	6576	8.3
	B	16	DVB–30 min	6625	2.9
		16	PTFE–30 min	6641	BQL
		16	DVB–60 min	6690	BQL
		16	PTFE–60 min	6706	BQL
	D	5	Neoprene	7089	11.0
		14	Butyl	7098	BQL
		19	Latex	7103	BQL
		25	Butyl	7109	33.2
		31	Latex	7115	BQL
		40	Neoprene	7124	BQL
	J	16	DVB–1st extraction	7036	BQL
		16	PTFE–1st extraction	7042	BQL
		16	DVB–2nd extraction	7084	BQL
	K	5	Latex	7188	BQL
		19	Butyl	7202	BQL
		25	Neoprene	7208	BQL
		28	Neoprene	7211	BQL
		31	Latex	7214	BQL
		40	Butyl	7223	BQL

BQL, below quantification limit.

Table 49. Individual Negative-Control Sample Results: Validation

Test Type	Test ID	Position No.	Material	Sample No.	Result (ng)
Validation	E	4	Latex	7528	BQL
		4	Latex–vapor	7567	BQL
		23	APC01	7547	BQL
		23v	APC01–vapor	7573	BQL
		30	APC01	7554	BQL
		30v	APC01–vapor	7576	BQL
		33	Latex	7557	BQL
		33v	Latex–vapor	7577	BQL
	F	5	Latex	7663	BQL
		5v	Latex–vapor	7702	BQL
		19	APC01	7677	BQL
		19v	APC01–vapor	7708	BQL
		25	APC01	7683	BQL
		25v	APC01–vapor	7711	BQL
		38	Latex	7696	BQL
		38v	Latex–vapor	7715	BQL
	H	5	Latex	7720	BQL
		28	Latex	7743	93.7
	M	14	Latex	7840	BQL
		25	Latex	7851	BQL
		38	Latex	7864	BQL
	N	9	Latex	8196	BQL
		9v	Latex–vapor	8233	BQL
		15	Latex	8202	BQL
		15v	Latex–vapor	8236	BQL
		17	APC01	8204	BQL
		17v	APC01–vapor	8237	BQL
		30	APC01	8217	BQL
		30v	APC01–vapor	8240	BQL

BQL, below quantification limit.

Some of the negative-control samples contained quantifiable levels of contaminant. This was especially true for the efficiency studies. The values from the 60 min extraction were changed from the 30 min extraction, suggesting that this may have been carryover in the analytical train. Low levels were occasionally noted in the solvent blank samples, supporting this hypothesis. As the program progressed, the negative-control samples were analyzed separately from the other samples to ensure they were accurate measures of cross-contamination within the laboratory test process. The change in process produced negative-control samples below the quantification limit during the later testing.

One negative-control sample studied during validation testing also had a measured value. This was attributed to potential process error. For every sample, a fresh set of disposable forceps was used to place the DVB pad into the extraction solvent. However, nondisposable metal forceps were used to remove the PTFE disk and the contaminated swatch from the DVB. This was necessary because the disposable forceps do not provide the fine control needed for this step. This was likely the source of the cross-contamination. A corrective action was implemented to use two metal forceps, one for the highly contaminated PTFE disk and another for the edge of the swatch.

5.4.2 Positive Controls

Samples known to provide measureable analytical responses were used to document that the test process was working properly. Statistics of multiple positive-control sample replicates were used to document the standard deviation in the test method. Test materials used for characterization down-selection and validation testing, such as latex and neoprene, were in this category.

5.4.3 Spike Controls

PTFE samples spiked with 6 µL of VX were used at the beginning and end of all neat tests to demonstrate operator proficiency and proper operation of the spiking device during that test. The results are included as part of the VX purity summary of Table 47.

5.4.4 Vapor Characterization Controls

As requested by JPM P, a limited number of vapor characterization controls were included in the validation tests. One of the 40 vapor characterization samples tested during the validation phase had a quantifiable mass of VX present. The concentration was near the limit of detection and yielded a total mass of 16.9 ng. This may have been cross-contamination from sample handling. A previous vapor characterization trial with that stainless steel weight did not have measurable VX in the sample. The comprehensive vapor characterization test results are shown in Table 50 for the 24 h validation tests and Table 51 for the 48 h validation tests. These results are separate from those collected during verification (see Section 3.9.4).

Table 50. Comprehensive Vapor Characterization Sample Results Obtained during 24 h Validation Testing

Contact Time (h)	Test ID	Position No.	Material	Sample No.	Result (ng)
24	E	1	Latex	7566	BQL
		4	Latex–NC	7567	BQL
		5	APC01	7568	BQL
		10	Latex	7569	BQL
		14	Latex	7570	BQL
		17	Latex	7571	BQL
		20	Latex	7572	BQL
		23	APC01–NC	7573	BQL
		25	APC01	7574	BQL
		28	APC01–NC	7575	BQL
		30	APC01	7576	BQL
		33	Latex–NC	7577	BQL
		35	APC01	7578	BQL
		36	Latex	7579	BQL
		40	APC01	7580	BQL
	F	3	Latex	7701	BQL
		5	Latex–NC	7702	BQL
		6	APC01	7703	BQL
		9	Latex	7704	BQL
		13	Latex	7705	BQL
		16	Latex	7706	BQL
		18	APC01	7707	BQL
		19	APC01–NC	7708	BQL
		21	APC01	7709	BQL
		22	APC01	7710	BQL
		25	APC01–NC	7711	BQL
		26	Latex	7712	BQL
		29	APC01	7713	BQL
		32	APC01	7714	BQL
		38	Latex–NC	7715	BQL
	N	1	APC01	8230	BQL
		3	Latex	8231	BQL
		5	APC01	8232	BQL
		9	Latex–NC	8233	BQL
		11	Latex	8234	BQL
		12	APC01	8235	BQL
		15	Latex–NC	8236	BQL
		17	APC01–NC	8237	BQL
		22	APC01	8238	BQL
		23	Latex	8239	BQL
		30	APC01–NC	8240	BQL
		33	Latex	8241	BQL
		38	Latex	8242	BQL
		39	APC01	8243	BQL

BQL, below quantification limit.
NC, negative control.

Table 51. Comprehensive Vapor Characterization Sample Results Obtained during 48 h Validation Testing

Contact Time (h)	Test ID	Position No.	Material	Sample No.	Result (ng)
48	H	9	Butyl	7724	BQL
		11	Butyl	7726	BQL
		26	Butyl	7741	BQL
		33	Butyl	7748	16.9
		35	Butyl	7750	BQL
	M	10	Butyl	7836	BQL
		16	Butyl	7842	BQL
		19	Butyl	7845	BQL
		26	Butyl	7852	BQL
		29	Butyl	7855	BQL

BQL, below quantification limit.

5.4.5 Preconditioning Chamber Logging

The environmental conditions within the preconditioning chamber were recorded during testing. The same controls required for the verification characterization were required during every test.

During preconditioning for Validation Test 2, Test F, the temperature-control requirements were not met. The average temperature was 33.5 °C, which was outside the required temperature. However, the absolute humidity requirements were met, indicating that the target level of moisture was present. The APC01 swatches from Test F were disqualified because of the lack of temperature control. However, the data was included in the report for completeness and to support discussion regarding the effects of environmental conditions on test data.

The preconditioning summary data for the validation testing is shown in Table 52. The temperature histograms are shown in Figure 35, and the temperature-time profile plots are shown in Figure 36. The RH histograms are shown in Figure 37, and the RH–time profile plots are shown in Figure 38. The absolute humidity histograms are shown in Figure 39, and the absolute humidity–time profile plots are shown in Figure 40.

Table 52 and Figures 35–40 fulfill the reporting requirements for environmental preconditioning.

Table 52. Preconditioning Data Summary: Validation Testing

Test	Temperature			RH			Absolute Humidity		
	Average (°C)	StDev (°C)	RSD (%)	Average (%)	StDev (%)	RSD (%)	Average (g/m^3)	StDev (g/m^3)	RSD (%)
Validation 1 Test E	32.32	0.13	0.41	80.09	1.06	1.32	27.54	0.49	1.78
Validation 2 Test F	33.53	0.70	2.10	77.31	1.00	1.30	28.35	0.88	3.12
Validation 3 Test N	32.08	0.27	0.84	81.39	3.40	4.17	27.63	1.13	4.10

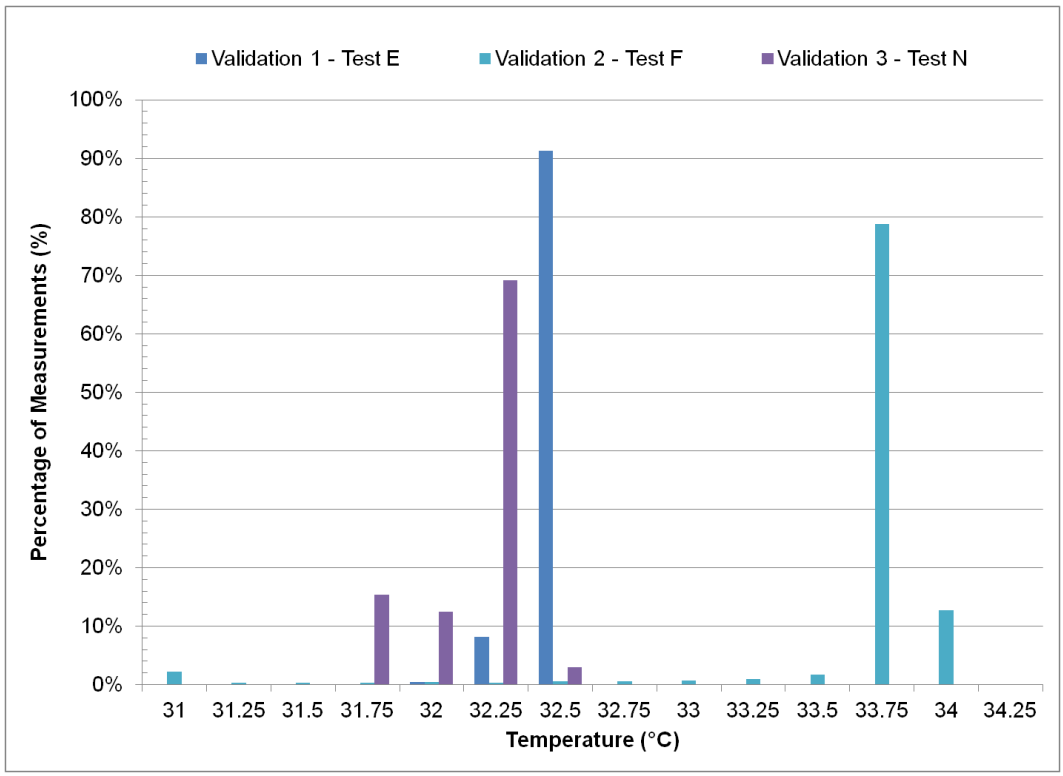


Figure 35. Preconditioning temperature histograms for validation testing.

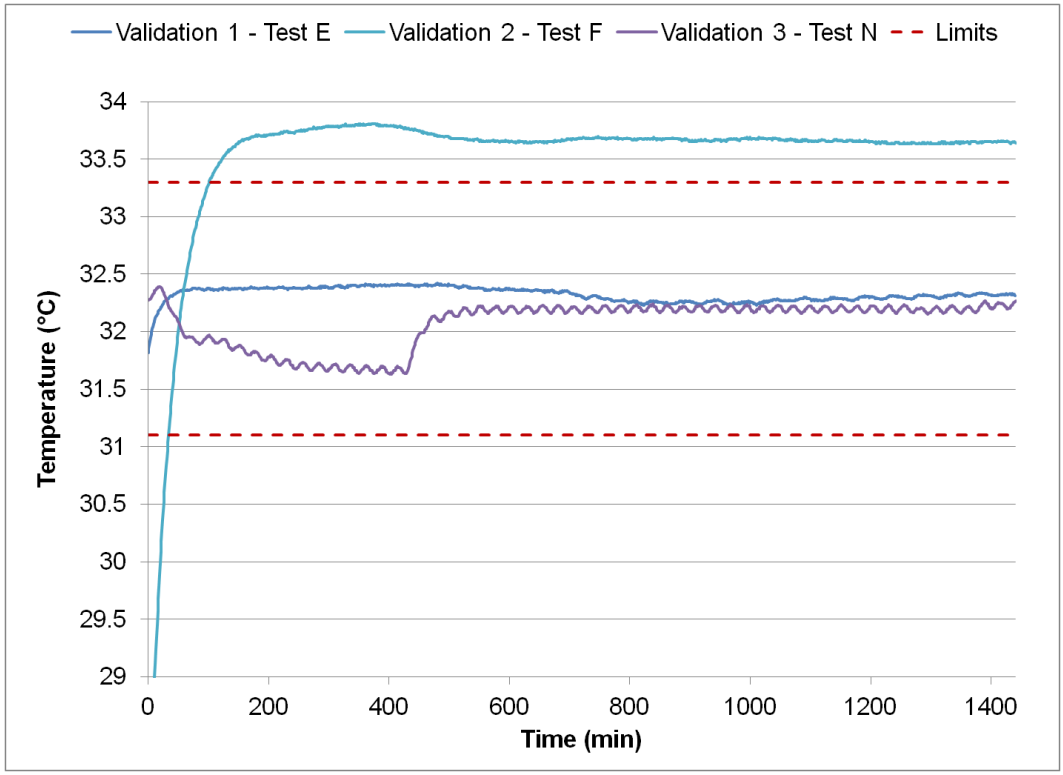


Figure 36. Preconditioning temperature–time profile plots for validation testing.

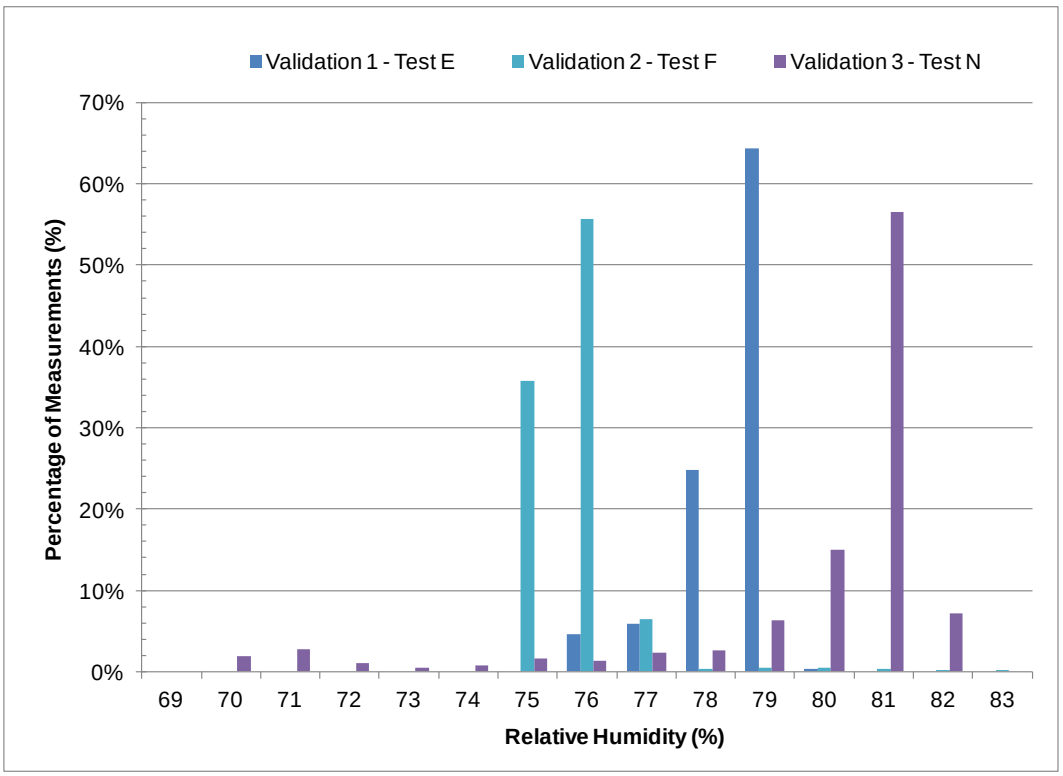


Figure 37. Preconditioning RH histograms for validation testing.

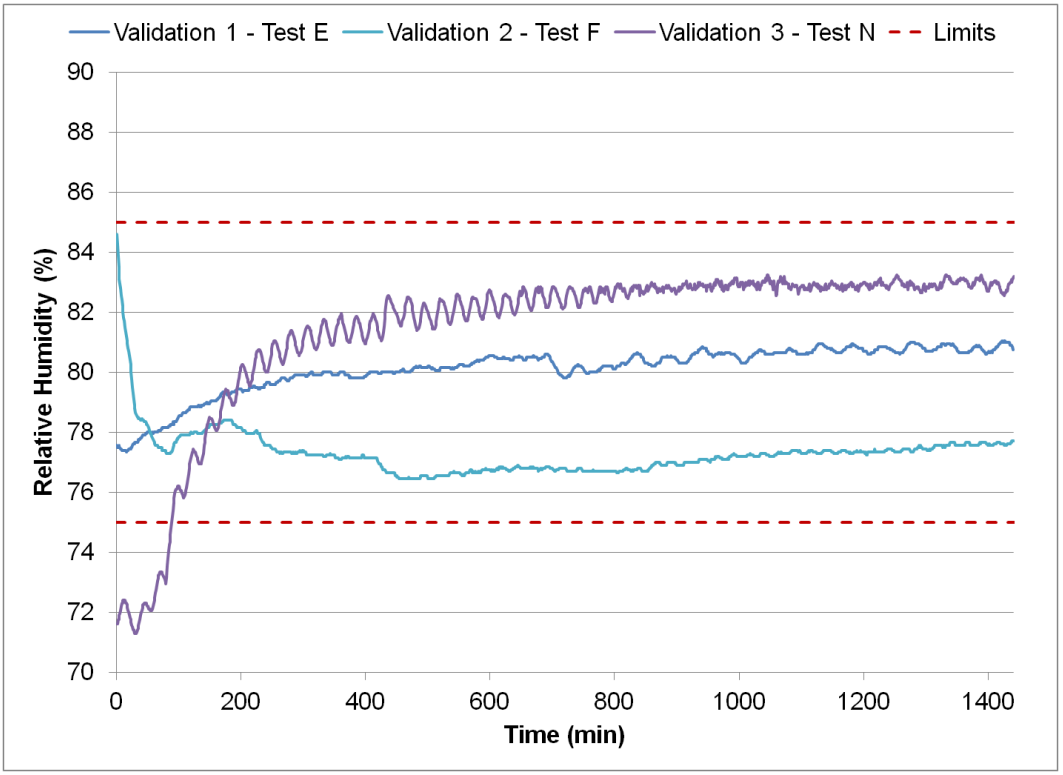


Figure 38. Preconditioning RH–time profile plots for validation testing.

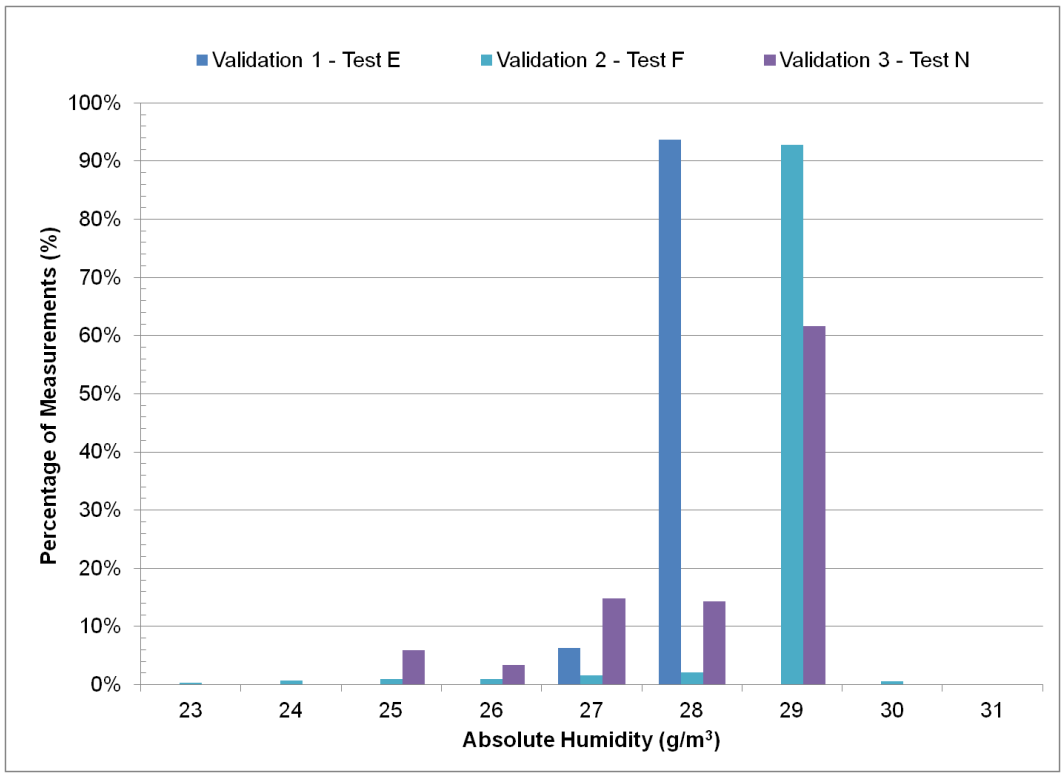


Figure 39. Preconditioning absolute humidity histograms for validation testing.

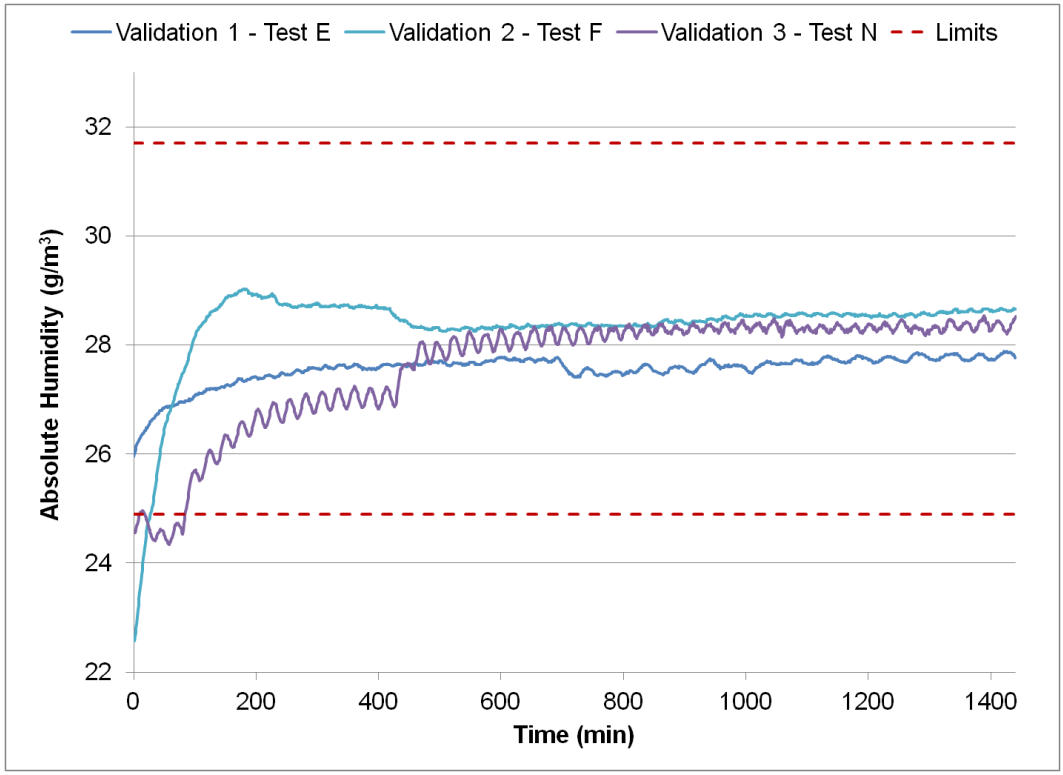


Figure 40. Preconditioning absolute humidity–time profile plots for validation testing.

5.4.6 Environmental Chamber Logging

The environmental conditions within the environmental test chamber incubator were recorded during testing. The same environmental controls required for the verification characterization were required during every test.

The environmental log for each verification test was compiled and documented in two ways. A histogram plot for the relative percentage of measurements for each temperature is provided in Figure 41. The temperature profile versus time for each test is provided in Figure 42 for the 24 h verification tests and Figure 43 for the 48 h verification test.

For the validation testing, the histogram plots are shown in Figure 44, and the temperature profile versus time is shown in Figure 45. Note that the temperature-logging computer stopped working part way through Test F. The data is shown is what was collected. There was no loss of temperature control during the rest of the test, only the loss of logging capability. Additional information is in Section 5.7.

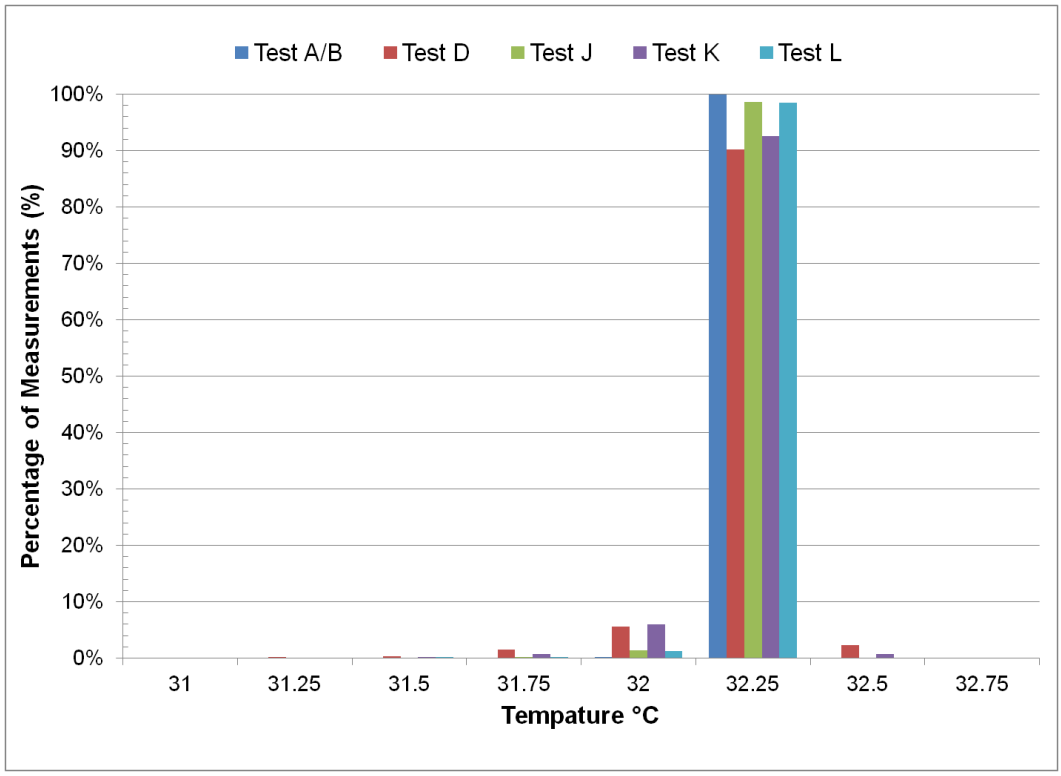


Figure 41. Temperature histogram for each verification test.

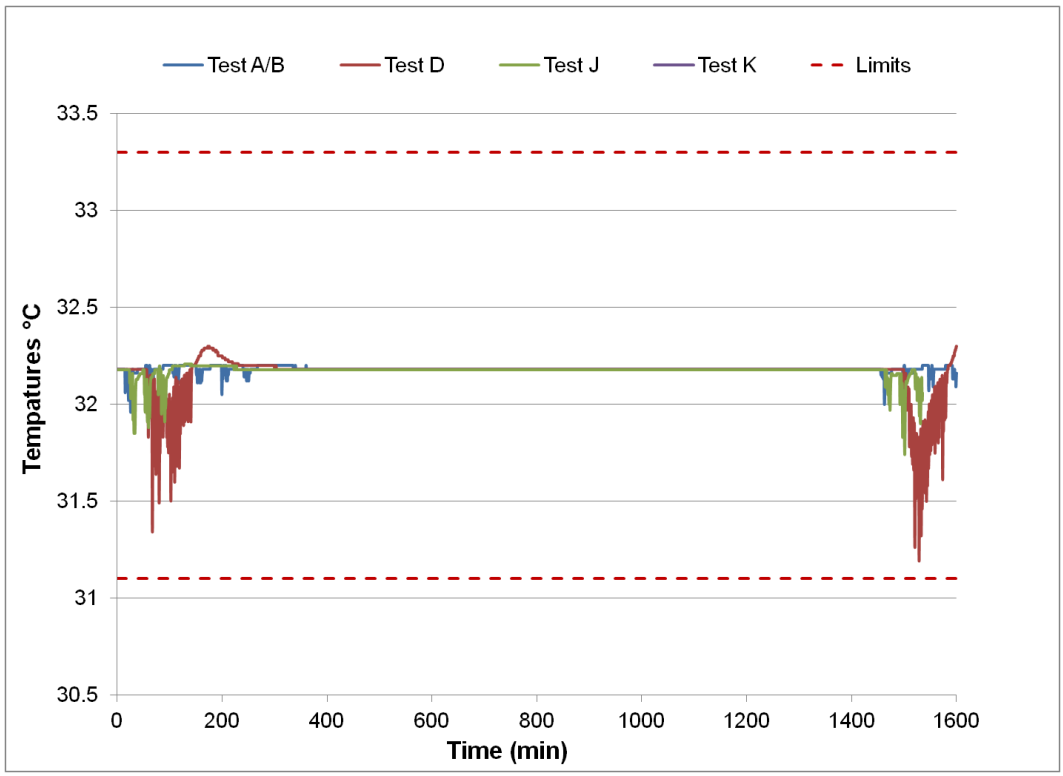


Figure 42. Temperature–time profile plot for 24 h verification test.

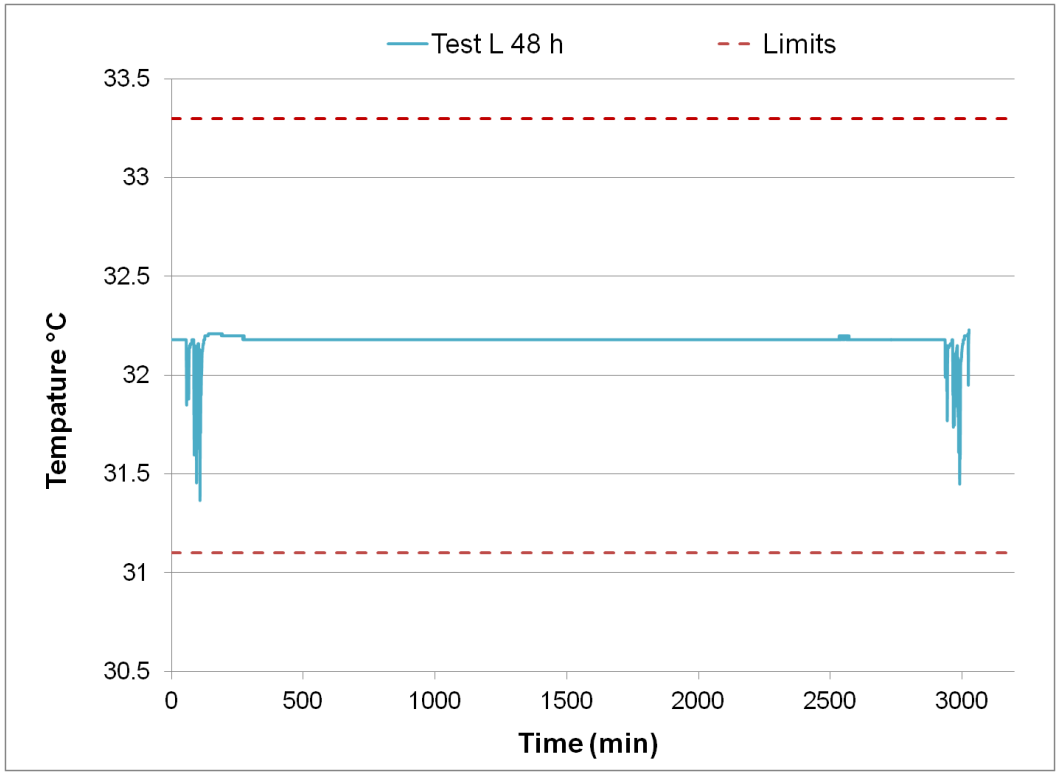


Figure 43. Temperature–time profile plot for 48 h verification test.

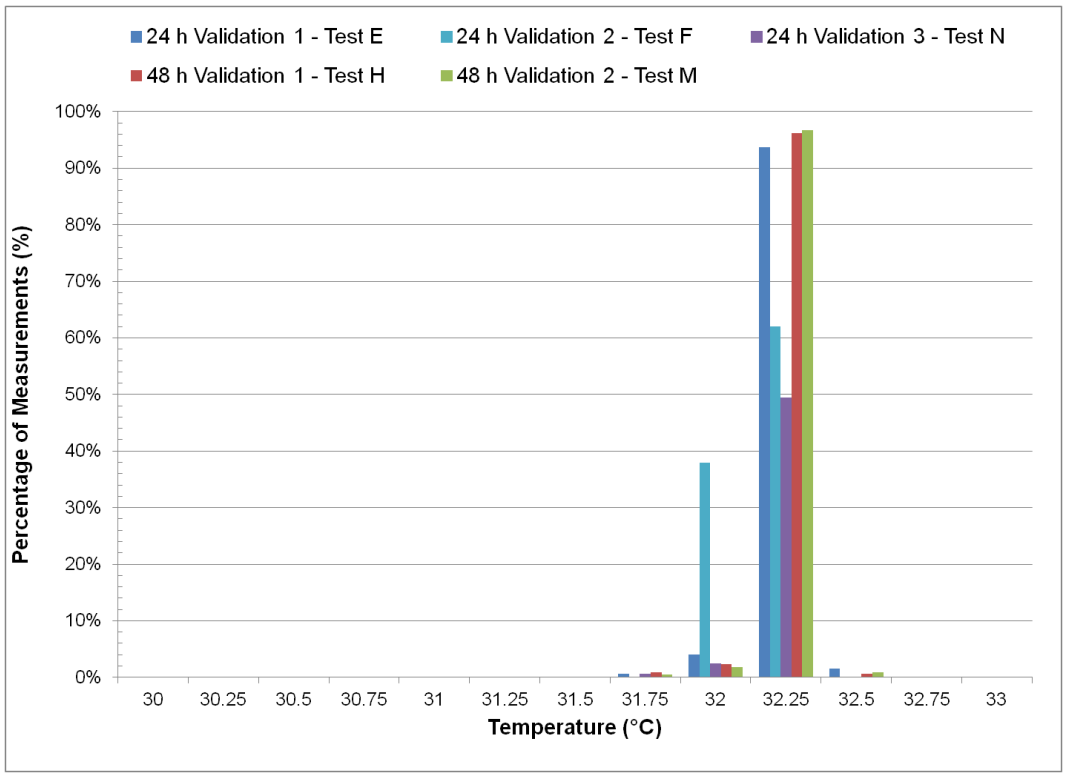


Figure 44. Temperature histogram plots for all validation tests.

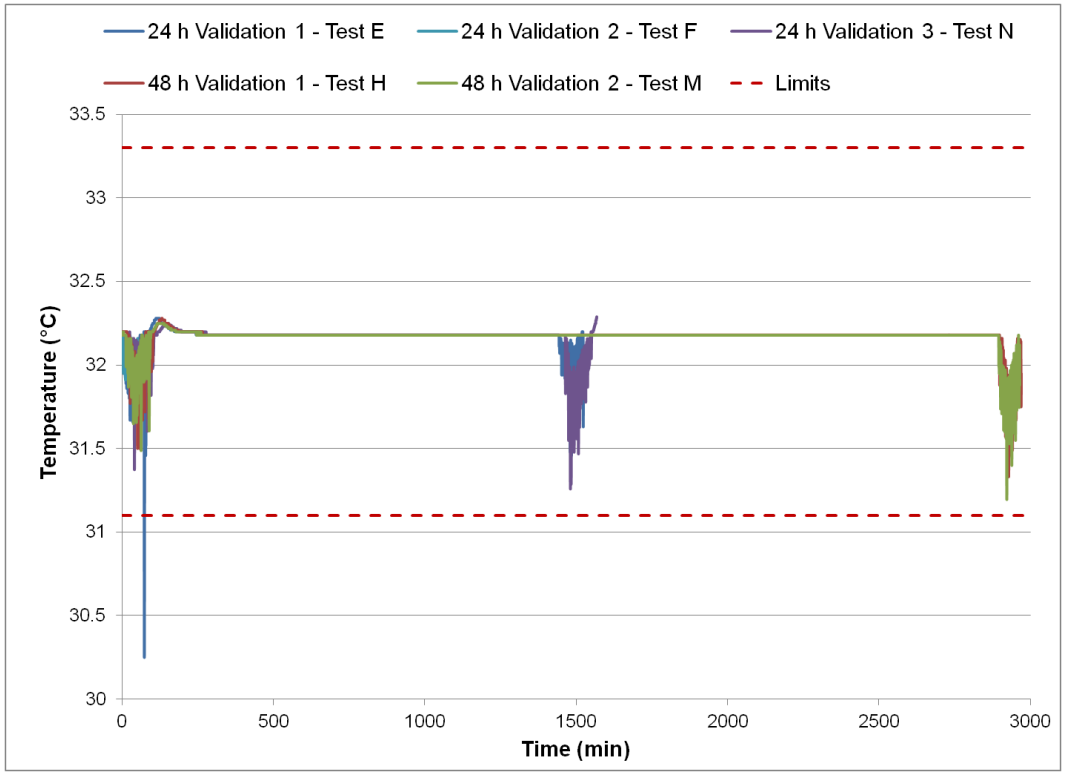


Figure 45. Temperature–time profile plots for all validation tests.

5.5 Run Sheets

Run sheets were developed as part of the experimental design to reduce sample bias. Each run sheet listed the test cell number, position, material, and individual sample identification. Positive and negative controls were designated on the run sheet. The time for each event that occurred during testing was recorded along with any observations. Each run sheet included a cover sheet with pertinent test information. Scanned copies of the run sheets are included in Appendix A.

5.6 Instrument Calibration

All instrumentation used during testing, such as temperature and RH indicators, analytical balances, etc., were NIST traceable and were within the current calibration interval. Items that required verification prior to use (e.g., analytical balance) were performance-verified using NIST-traceable, calibrated reference standards. Analytical instrumentation, including the LC-MSMS, was calibrated prior to use using procedures outlined in the PASB Quality Management System. A listing of the calibrated equipment used during the test program is provided in Table 53 and includes manufacturers, model and serial numbers, and calibration dates.

Table 53. Calibrated Instrumentation for Temperature, Humidity, Mass, and Swatch Thickness Measurements

Brand (Location) Equipment Type	Serial No.	Calibration Date	Void Date	Test Used	Position or Location
Omega (Stamford, CT) OM-CP-TEMP101 data logger	N40884	27-Feb-13	22-Feb-14	Incubator characterization	Top back left
	N40825	28-Feb-13	23-Feb-14	Incubator characterization	Top back right
	N40889	26-Feb-13	21-Feb-14	Incubator characterization	Top center
	N18853	18-Mar-13	13-Mar-14	Incubator characterization	Top front left
	N18829	21-Mar-13	16-Mar-14	Incubator characterization	Top front right
	N18831	22-Mar-13	17-Mar-14	Incubator characterization	Bottom back left
	N18833	21-Mar-13	16-Mar-14	Incubator characterization	Bottom back right
	N40874	13-Aug-13	8-Aug-14	Incubator characterization	Bottom center
	N40867	13-Aug-13	8-Aug-14	Incubator characterization	Bottom front left
	N18832	22-Mar-13	17-Mar-14	Incubator characterization	Bottom front right
Fisher Scientific (Waltham, MA) 15-077-976 thermometer	122500188	As received	1-Sep-14	All tests with incubator	Bottom center
	130610809	As received	15-Oct-15	Incubator temperature comparison	Bottom center
Omega/ OM-CP-RHTEMP101A data logger	P34557	21-Mar-14	21-Mar-15	Preconditioning characterization	Front lower left
	P295571	25-Feb-14	25-Feb-15	Preconditioning characterization	Back upper right
Vaisala (Vantaa, Finland) HM70 meter	F0930013	30-Jul-13	25-Jul-14	Preconditioning characterization	Inlet reading
Vaisala HMI41 meter	C2630013	4-Feb-13	30-Jan-15	Preconditioning characterization	Outlet reading
Sartorius (Goettingen, Germany) IB16000S balance	39040007	6-Feb-13	1-Feb-14	Stainless steel mass measurement	n/a
Troemner (Thorofare, NJ) UltraClass mass standard	40000011011	10-Jul-12	25-Jul-15	Mass verification	n/a
Mettler Toledo (Toledo, OH) mass standard	80126	13-Oct-11	27-Sep-14	Mass verification	n/a
Mitutoyo (Kanagawa, Japan) micrometer	5210-00J02	3-Jun-13	18-May-16	Stainless steel dimensional measurement	n/a
Mitutoyo 516-935-26 gage block set	1206919	27-Sep-12	27-Sep-15	Thickness verification	n/a
Mitutoyo 547-500 thickness gage	13104050	16-Jan-14	16-Jan-15	Thickness measurement	n/a
Mettler Toledo balance	1129400088	4-Dec-13	29-Nov-14	Mass measurement	n/a
Troemner UltraClass weight set	77240	20-Apr-12	5-Apr-15	Mass verification	n/a

n/a, not applicable.

5.7 Deviations and Corrective Actions

Several deviations were noted during the verification trials. The observations, impacts on testing, and remediation methods were provided for each instance as appropriate.

Analytical Instrument

The lowest-concentration calibration curve standard (0.118 ng/mL) was higher than the target for five of the seven replicates. Some of the results were outside the target range of ±20%. This was attributed to carryover between analyses. This was not expected to affect testing, as smaller dynamic ranges were used, and the individual results from each calibration curve tested passed the accuracy requirements.

Preconditioning

(1) During verification of the preconditioning chamber operation, the device measuring and logging the outlet conditionings stopped working 16 h into the trial. It was likely that the device was in need of a new battery. This instance did not impact testing: the conditions within the preconditioning chamber remained constant and within required specifications, as measured by logging devices co-located with the swatches. A new device was ordered to measure and log the outlet conditions.

(2) During the swatch-drying portion of the preconditioning trials, the instrument logging the inlet conditions stopped working partway through the testing. The reason for this malfunction was not determined. This instance had no effect on the trial: the conditions within the conditioning chamber remained constant. The inlet-monitoring device has been checked for proper function. However, its functionality does not affect the ability to control the chamber or monitor the swatch location.

(3) During swatch conditioning at 32.2 °C and 80% RH, the inlet temperature was higher than the target. This was thought to be caused by the preconditioning chamber being located near the hot-air recirculator in the environmental control chamber. This instance had no effect on the trial: the conditions within the conditioning chamber remained constant and within the required specifications as measured by logging devices co-located with the swatches. The inlet-monitoring device was checked for proper function. However, its functionality does not affect the ability to control the chamber or monitor the swatch location.

Uptake and Extraction Efficiency

(1) In the test plan, it was stated that sample spikes would be separated by 2 min to allow for sample breakdown and aliquot collection. In some instances, the PTFE and DVB pads were spiked with 1 min separations. This change occurred because it was not always necessary to wait 2 min to allow for sample collection. The times were noted on the run sheet. This point was noted for completeness. No corrective or remedial actions were necessary.

(2) During the initial uptake efficiency test with a 10 mL acetonitrile extraction, some of the samples may have been inadvertently mislabeled. This affected the 200 ng condition of the uptake efficiency testing. These samples are marked with an asterisk in Table 25. This did not have an overall effect on the verification because acetonitrile was no longer to be used for extraction, and 20 mL was chosen for the extraction volume. As a remedy, timing charts that included clearer sequential prompts for samples were developed for tests that included overlap of aliquot timings.

(3) During the 48 h uptake and extraction efficiency testing, the aliquots for five samples were pulled late. As a result, these samples were in the extraction solvent for an additional 5 min. The

samples affected were the 2000 ng DVB extraction efficiency samples, with a 1 min contact prior to extraction. The additional extraction time did not affect the testing. This was demonstrated in two ways. First, no difference was observed when the 30 and 60 min extraction times were compared, as was evaluated during Test A. Second, the results for these samples were consistent with those from the other 1 min contact-period samples at other concentrations.

Verification Testing

(1) During the initial characterization verification, Test D, the operators neglected to put a foil barrier under the butyl swatches. The latex and neoprene results were not affected. The test was performed again and labeled as Test K. The operators were reminded to carefully read the test plan prior to beginning operations.

(2) During Test K, one of the samples was rerun with a different dilution. The QC samples did not meet the minimum requirements. This was likely caused by carryover from a previous analytical queue. Unfortunately, the original sample was lost before a new dilution and sample could be obtained. This sample was marked as lost. Because the sample data was not used, the analytical QC data was not included in the summary statistics (Section 3.4).

Validation Testing

(1) Within the test plan, a typographical error was noted in eq 4: the d and t were inverted. This equation was used to calculate the power statement for the validation testing. However, the calculation had been performed correctly, so the typographical error did not affect the results. This error is noted here for completeness. The correct equation should read as follows:

$$s = \sqrt{\frac{d^2 n}{t^2}} \tag{4}$$

(2) During validation testing, the calibration curve was adjusted to have a range from 0.52 to 520 ng/mL. As described in Section 3.4, the lowest-concentration calibration curve standard (0.118 ng/mL) was higher than the target for five of the seven replicates. Some of the results were outside the target range of ±20%. This was attributed to carryover between analyses. The smaller dynamic range helped focus the instrument on the concentration of the samples being analyzed and was a necessary work-around due to carryover that affected the precision of the 0.118 ng/mL standards. This adjustment was noted within the verification report;[12] however, it was a deviation from the test plan. This was not expected to affect the overall method.

(3) During the first 24 h validation test, the operators did not measure the thickness of the latex swatches. All of the swatches came from the same roll of latex, which was received with a certificate of conformance with the specifications. Furthermore, all of the thickness measurements were very consistent, as shown in Table 46. The corrective action was to obtain triplicate measurements from 10 locations of the bulk latex sheet from the region where swatches for this test were taken (Figure 34). These measurements were within the standard deviation of the other thickness measurements. Furthermore, the permeation measurements for this test were also within the standard deviation for those for the other 24 h latex samples. Therefore, it was concluded that the thickness of this region of latex was within the acceptable range, and the lack of thickness measurements for each swatch did not affect the test results.

(4) During the second 24 h validation test, the temperature requirements were not met in the preconditioning chamber. This issue was not discovered until after the test was completed. The

temperature plot and permeation results are included in this report to facilitate future discussion regarding the effect of temperature on permeation. The lack of temperature control did not seem to influence the final permeation numbers. This was thought to be because the absolute humidity (i.e., total water moisture mass) was controlled within the requirements, suggesting that water content had a greater influence than preconditioning temperature on APC01 permeation. The corrective action was to repeat the testing.

(5) During the second 24 h validation test, the test chamber log did not operate properly. There was no indication that loss of temperature control occurred during this period. The temperature display on the front of the test chamber indicated that the temperature was in range, even when the door was opened and closed to remove the individual samples at the end of testing. This issue with the temperature log did not affect the permeation test results. The corrective action was to periodically check that the logging system was collecting data while the operators were in the room. The logging system worked properly during subsequent tests.

(6) During the first 48 h validation test, one of the negative-control samples had measureable levels of VX. This was attributed to potential process error. A fresh set of disposable forceps was used for every sample to place the DVB pad into the extraction solvent. However, nondisposable metal forceps were used to remove the PTFE disk and contaminated swatch from the DVB. These were necessary because the disposable forceps do not allow the fine control that is needed for this step. It is thought that the cross-contamination occurred there. A corrective action was implemented whereby two metal forceps were used: one for the highly contaminated PTFE disk and another for the edge of the swatch. In subsequent testing, no cross-contamination to negative controls occurred.

(7) During the first 48 h validation test, one of the butyl vapor control samples contained measureable levels of VX. This was attributed to potential process error. As mentioned in point (6) above, nondisposable forceps, which allow for fine control in handling, were used to remove the PTFE disk and contaminated swatch from the DVB. These forceps were likely the source for the cross-contamination during this validation test. A corrective action was implemented whereby two metal forceps were used: one for the highly contaminated PTFE disk, and another for the edge of the swatch. This vapor sample was processed several swatches after the negative-control sample that exhibited cross-contamination, and its VX level was significantly lower than that for the negative control. This further supports the theory that a cross-contamination event had occurred because use of additional forceps would remove the cross-contamination.

(8) During the third 24 h validation test, Test N, the certification of the NIST-traceable thermocouple in the environmental test chamber had expired. It was originally expected that testing would be concluded prior to the expiration of the thermocouple. The temperature reading of the expired thermocouple was compared to a within-calibration NIST-traceable thermocouple to verify the performance of the original thermocouple. The expired thermocouple was operating within the calibration specifications. Therefore, the temperature data from Test N was considered valid. The corrective action was to replace the expired thermocouple with a new one.

Statistical Analysis

The original plan for addressing permeation levels below the quantitation limit was to use one-half of the quantitation limit as a substitution, followed by standard statistical analysis. Since the approval of the test plan, a more robust method was identified and used. Within this report, the quantitation limit was used as a substitution, and the statistical comparison was made using a nonparametric Wilcoxon test.[14] This case only applied to the characterization testing of butyl swatches tested with O-ring gaskets in Section 3.9. Although more robust and statistically correct, this method represents a change from the original test plan, and it is noted here as a deviation.

6. STATISTICAL ANALYSIS

Appropriate statistical analyses were performed to make comparisons between the data sets and determine whether the differences between the means were statistically relevant.

6.1 Student's *t* Test and Welch's *t* Test

A standard statistical approach for comparing two data sets is the Student's *t* test. When this method was used, it was assumed that the data sets were normally distributed, had equal variances, and were independent. For cases where the variances were not equal, the more complex Welch's *t* test was used.

Both approaches return a *p* value, which is used to determine whether the means of the two data groups are statistically different. The *p* value is the probability of obtaining a result at least as contradictory to the null hypothesis just by chance if the null hypothesis was in fact true. The *p* value indicates whether there is sufficient evidence to reject the null hypothesis. The null hypothesis states that the mean value is the same for both data sets. A large *p* value indicates the there is insufficient evidence to reject the null hypothesis, and therefore, the data sets are not statistically different. A *p* value less than the α value, typically 0.05, indicates that it is unlikely that the difference between data set mean values is the result of the coincidence of random sampling. This is sufficient evidence to reject the null hypothesis and accept that the data sets have mean values that are statistically different from each other.

6.2 Censored Data and Data Transformations

The requirement for data transformation was dependent on the distribution of the results. Examples included normal and log-normal distributions.

Permeation testing resulted in analysis of a contaminant within a sample extract. Because of sample, material, and test method variability, some studies may have resulted in a standard deviation that was greater than the mean value. Such data sets would have indicated that the data distribution could include negative values. However, it would be impossible to have a negative quantity of contaminant because this would not be physically realistic. Therefore, such data would not have a normal distribution and would require transformation to meet the requirements for a particular statistical analysis test. Because the data was required to be greater than or equal to zero, it was considered to be left-censored data. Left-censored data would be managed using a log transformation, which would remove the issue of negative numbers.[15]

However, none of the measured permeation values had standard deviations greater than the mean. Therefore, the choice of whether to use a data transformation was based on the data distribution. Here, the data was normally distributed, and no transformation was required.

6.3 Permeation Levels Below the Quantification Limit

Permeation levels below the analytical quantification limit were listed as "BQL" in all tables, which stood for *below quantification limit.* When a sample below the analytical quantification limit was used for statistical calculations, a value of the quantification limit was used as a substitution, and the analysis followed the nonparametric process of a Wilcoxon test.[14] This case only applied to the characterization testing of butyl swatches tested with O-ring gaskets in Section 3.9.

6.4 Calculating the Single-Laboratory Standard Deviation

Standard statistical methods were used to calculate the single-laboratory precision for the LVAP test method. An example is the intermediate-precision standard deviation (IPSD) method, detailed in ISO 5725-3:1994.[16] The IPSD method was used to calculate the standard deviation of the method when executed by a single laboratory, where certain parameters were held constant and others were allowed to vary. Parameters held constant were the laboratory, operators, and test equipment. Parameters allowed to vary were the test day and the analytical calibration curves, because a new calibration curve was generated for each test day. The IPSD was calculated for both the air-permeable and air-impermeable materials.

The calculations may be expanded to include data from additional laboratories as it becomes available.

6.4.1 Definitions

To facilitate discussion of the standard deviation of the test method, the definitions of the specific technical terms are provided here, as they apply to this test.

Repeatability (S_r): The standard deviation of responses for measurements made under repeatability conditions. S_r was the within-test-day standard deviation. Repeatability conditions are multiple responses from within the same test day, where all aspects remain constant between measurements with regard to operators, laboratory, equipment, and calibration. The S_r calculated for this study was generated using only a single laboratory and is therefore referred to as a "single-laboratory" S_r to distinguish it from the more comprehensive S_r estimate that may be obtained from multi-laboratory studies.

Between-test-day standard deviation (S_L): The between-test-day standard deviation for measurements made on different test days. The S_L for this single-laboratory study was representative of changes to test day and calibration. The conditions that remained constant between test days were the operator and test equipment. The S_L accounts for variability attributable to changes in testing from day to day. The S_L does not account for variability within the same test day, such as random error.

Intermediate-precision standard deviation (IPSD): The standard deviation of responses for measurements made under IPSD conditions. Under IPSD conditions, some factors are allowed to vary, but the laboratory remains constant. Conditions that remained constant were the operator and test equipment. The IPSD accounts for variability from within a single test day and day to day, based on the following relations:

$$IPSD = \sqrt{\left(S_r^2 + S_L^2\right)} \tag{5}$$

Reproducibility (S_R): The standard deviation of responses for measurements made under reproducibility conditions. Reproducibility conditions require measurements from different laboratories. The data in this V&V report is from a single laboratory; therefore, S_R is not applicable, and the term "reproducibility" is not used.

6.4.2 Calculations

The validation data was technically consistent with the ISO 5725 procedures for estimation of IPSD as described in ISO 5725-3,[16] Section 8.0, "Within-Laboratory Study and Analysis of Intermediate Precision Measures", subsection 8.2, "An Alternative Method". It should be noted that the

number of test days for each condition constituted a very small sample size that was lower than the typical number of replicates used for an IPSD study.

The factors of time (different test days) and calibration (different analytical calibration curves) were varied during the study, whereas equipment and operators were not changed. In ISO 5725 terminology, the IPSD would be labeled as $IPSD_{(TC)}$, with the subscript referring to time and calibration. Other factors, including ambient atmospheric conditions and other background conditions, were not controlled. The agent-specific agent vial also changed during the course of the V&V. However, this change was not included as part of the experimental design. Additional information is provided in Section 7.

The formula number 11, provided in ISO 5725-3, Section 8.2.2 for the calculation of IPSD, required balanced data sets, with the same number of replicates used per day. This formula was not applicable to nonbalanced data sets because it was unable to account for variable degrees of freedom per test day. The formula number 11 and the other ISO 5725-3 formulas for the calculation of S_r, S_L, and S_R were derived from the basic statistical model given in ISO 5725-3, Section 6.1. This basic statistical model was a random-effects model with the laboratory, test day, operator, calibration, and equipment serving as random factors. Therefore, the precision estimates were calculated directly by fitting this random-effects model to the data using the residual maximum-likelihood (REML) method. The REML method was more appropriate than the expected mean squares (EMS) method described in ISO 5725-3 because the validation data set was unbalanced.[17] JMP 11 software (SAS Institute; Cary, NC) was used on the validation data to find the precision estimates (via REML methods) by calculating the variance components for the random-effects model. The precision estimates are given by the variance components after a random-effects model is fit using JMP 11 "Fit Model", with the test day designated as a random effect. For each validation set, the following equations were used within JMP 11, where the precision estimates were expressed as a percentage of the grand average of the response:

$$S_r\,(\%) = \frac{\sqrt{Residual\ Variance\ Component}}{Grand\ Average\ of\ the\ Response} \times 100 \tag{6}$$

$$S_L\,(\%) = \frac{\sqrt{Test\ Day\ Variance\ Component}}{Grand\ Average\ of\ the\ Response} \times 100 \tag{7}$$

$$IPSD\,(\%) = \frac{\sqrt{Total\ Variance\ Component}}{Grand\ Average\ of\ the\ Response} \times 100 \tag{8}$$

6.4.3 Statistical Outliers and IPSD Results

Regarding outlier data, the IPSD was calculated twice. ISO 5725-3 guidance was to use a Grubbs method to remove statistical outliers. The outlier data points were flagged in the validation data tables, Tables 39–41. These data points were statistical outliers, given that no attributable cause for removal was noted in the run sheet during testing. This approach was used to maintain compliance with the ISO method, and the results are provided in Table 54. The estimates are in terms of percentage of the average response. The table also has an additional row that includes the results for a third test day with APC01. The included test (Test F) did not meet the preconditioning temperature requirement, but did meet the preconditioning absolute humidity requirement, which indicated that the moisture requirement for the carbon had been met.

Given the limited number of test days, and at the request of the IP CAPAT, the IPSD was also calculated with all data included. Here, the standard deviation was larger because more extreme data

points were included, such as the APC01 result that was approximately 6 times higher than the mean. The results are provided in Table 55. The estimates are in terms of percentage of the average response.

Table 54. LVAP-Calculated IPSD for Single-Laboratory Testing: Outliers Removed

Material	Contact Time (h)	S_r (Repeatability) (%)	S_L (%)	IPSD (%)
PTFE control for dosing tools	n/a	1.2	5.8	5.9
APC01 2 test days	24	13.8	13.2	19.1
APC01 3 test days (includes test that did not meet preconditioning temperature requirements)	24	11.9	9.9	15.4
Latex	24	3.2	5.8	6.7
	48	3.6	1.6	4.0

n/a, not applicable.

Table 55. LVAP-Calculated IPSD for Single-Laboratory Testing: All Data

Material	Contact Time (h)	S_r (Repeatability) (%)	S_L (%)	IPSD (%)
PTFE control for dosing tools	n/a	1.2	5.8	5.9
APC01 2 test days	24*	83.6*	22.9*	86.8*
Latex	24	5.2	6.3	8.2
	48	4.6	2.1	5.0

n/a, not applicable.
*Includes extreme data point: see Section 4.3 for additional information.

6.4.4 Interpretation and Application of the Precision Estimates

The validation testing was conducted with two well-characterized and standard materials, latex and APC01. Using these materials, the IPSD represents the standard deviation of LVAP as a test method. The IPSD estimated for each material is interpreted to mean that samples collected under IPSD conditions (same laboratory but different days, calibration, etc.) would be expected to have a standard deviation of 8.2% for impermeable materials and 86.8% for air-permeable materials (19.1% if the single extreme outlier were removed). During testing of test swatches for programs, standard deviations beyond these estimates would be the result of variability in the material or, potentially, the result of a greatly reduced concentration regime, as described in Section 7.2.

7. CONTEXT AND DISCUSSION

This section provides context for the validation results by discussing potential sources of variance and how they may affect future programs that incorporate LVAP as a test method.

7.1 Effect of Multiple Agent Vials

A potential source of variability was the use of multiple agent vials of VX during testing. The requirement for this V&V was to use agent with >90% purity, measured during each test day. This requirement was met. One lot of VX (VX-U-1223-CTF-N) was used throughout the testing. However, several vials of VX from this lot were used throughout the course of the testing. As the contents of the vial were exhausted, a new vial was used. Although it was not part of the experimental design, there may be a correlation between the level of variability and the specific vial of VX used during the test. All verification tests were conducted using neat agent from vial 13. All verification test samples, including Tests D and K, were analyzed using stock standards generated from neat agent from vial 13. Validation tests were conducted using neat agent from vials 14, 15, 17, and 18. All validation test samples were analyzed using stock standards generated from neat agent from vial 14. The timeline linking the individual tests, the measured agent purity, and the VX neat agent vial is shown in Table 56.

Table 56. Timeline Linking Calibration Stock Standards, Individual Tests, and VX Vial Numbers

Date	Test Category	Test ID	Test Description	Average Purity (%)	VX Vial No.
5-Mar-2014	Analytical	n/a	New stock standards from neat agent	—	13
26-Mar-14	Verification	D	Characterization testing	91.6	
8-Apr-14	Verification	K	Characterization testing	91.1	
21-May-14	Analytical	n/a	New stock standards from neat agent	—	14
9-Jul-14	Validation	E	24 h Validation 1	94.5	
22-Jul-14	Validation	F	24 h Validation 2	100.8	15
29-Jul-14	Validation	H	48 h Validation 1	104.6	
18-Aug-14	Validation	M	48 h Validation 2	101.4	18
16-Sep-14	Validation	N	24 h Validation 3	103.7	17

n/a, not applicable.

The VX used throughout this test program met the performance requirement of >90% purity. Controlling for the VX vial was outside the scope of the V&V test program and would have required a more complex experimental design, including the use of multiple VX vials per test day. Because a single vial was used for each test day, any potential "vial effects" were confounded with the test day–calibration effect, and it was not possible to isolate and quantitatively measure the variability that may have been transmitted to the method's precision estimates solely as a result of purity differences between vials. Therefore, the combined effects of test day and agent vial differences were estimated by S_L.

7.2 Benchmark Comparison to Industry Validation Performance and the Effect of Concentration Regime on Variability

Beyond publishing the IPSD standard deviation of LVAP as a test method, it would be useful to benchmark the LVAP IPSD against the variability in other test methods. Such a comparison would provide greater context for LVAP as a test method with regard to expected variability measured in the broader testing world.

A method for conducting such a comparison is the Horwitz calculation. In 1980, William Horwitz conducted an empirical analysis of the results of over 50 method-validation studies involving analytical quantification. His analysis demonstrated that the resulting reproducibility, as determined by a method-validation study, can be predicted using only the mass-to-mass concentration of the analyte.[18] The predicted reproducibility standard deviation is given by the Horwitz formula:

$$\mathrm{PRSD_R}(\%) = 2 \times C^{-0.15} \tag{9}$$

where $PRSD_R$ (%) is the predicted relative reproducibility standard deviation expressed as a percentage of the average response of the method (this is a prediction of the value S_R), and C is the mass-to-mass concentration of the analyte. For the purpose of this evaluation, the PTFE spike samples were used for the calculations. Within the context of this V&V, the concentration would be the known VX mass divided by the mass of the 20 mL acetone extraction, providing the mass-to-mass concentration.

The $PRSD_R$ predicts the global reproducibility and serves as the overall benchmark level. Remarkably, eq 9 seems to hold true regardless of the type of analyte, the type of analytical method, or the era in which the validation study was performed. The database of method-validation studies used includes data from the early 1900s and has since been updated to include almost 10,000 individual validation studies.[19]

To compare the calculated standard deviation for a single method to the benchmark, the Horwitz ratio (HorRat) was devised, as defined by

$$\mathrm{HorRat} = \frac{\mathrm{RSD_R}}{\mathrm{PRSD_R}} \tag{10}$$

where RSD_R (%) is the relative reproducibility standard deviation calculated from the method-validation study and expressed as a percentage of the average response of the method. Within the context of this V&V, the repeatability, RSD_r, was used as a single-laboratory variant. $PRSD_R$ (%) is the predicted relative reproducibility standard deviation based on the mass-to-mass concentration of the analyte, as defined in eq 9.

The stipulations for the Horwitz formula and HorRat were that the analytical method must have a true "target" value (i.e., not a purely method-dependent response), and the method response must not be a physical property such as color, viscosity, or moisture content.[18] The Horwitz formulas were normally associated with multiple-laboratory method-validation studies and used "reproducibility" terminology (see Section 6.4.1). Because the data in this V&V was sourced from a single laboratory, the reproducibility RSD_R, and therefore the HorRat, could not be estimated. However, the single-laboratory variant, $HorRat_r$ using RSD_r, was calculated.

The PTFE spike samples may serve as an example of the calculation process. Here, 6 mg of VX was spiked onto PTFE and extracted in 20 mL of acetone. Given an acetone density of 0.79 g/mL, the mass-to-mass concentration of this solution was calculated as

$$C = \frac{0.006\ \mathrm{g\ VX}}{20\ \mathrm{mL\ acetone} \times 0.79\ \mathrm{g/mL}} = 0.00038\ \text{mass-to-mass concentration}$$

The $PRSD_R$ was calculated using eq 9:

$$\mathrm{PRSD_R} = 2 \times C^{-0.15} = 2 \times 0.00038^{-0.15} = 6.52\%$$

The $HorRat_r$ was calculated using eq 10, where the RSD_r was the S_r repeatability value for the PTFE spike sample, substituted for RSD_R:

$$HorRat_r = \frac{RSD_r}{PRSD_R} = \frac{RSD_r}{6.521} = \frac{1.2}{6.52} = 0.18$$

where RSD_r (%) is the relative repeatability standard deviation, S_r (%), calculated from the method-validation study and expressed as a percentage of the average response of the method.

The $HorRat_r$ at 0.18 for the spiked PTFE sample method was slightly better than the expected range of 0.3 to 1.3, indicating that the method slightly exceeded the performance expected for this analyte concentration regime, relative to the historical database of method validation.[20]

A similar treatment may be applied to the latex and APC01 results. Applying the Horwitz analysis to these other materials would require defining the average for these materials as "truth". Although it might not be possible to apply a formalized Horwitz analysis of the results, such an evaluation provides additional context regarding the cause of variability and may help explain an alternative cause for the differences in standard deviation between the material types. The HorRat was calculated for latex and APC01, and those values are provided in Table 57. For this calculation, statistical outliers were included and outliers with attribution were excluded.

Table 57. HorRat Benchmarking of the Method Variance Based on Concentration Regime: All Data

Parameter	Material			
	Spiked PTFE Sample	Latex		APC01
Time	—	24 h	48 h	24 h
Test days	7	5	2	2
Average measured mass (µg)	5892	4798	5324	16.41
C	3.72E-04	3.03E-04	3.36E-04	1.04E-06
$PRSD_R$ (%)	6.54	6.74	6.64	15.80
RSD_r (%)	1.2	5.2	4.6	83.6
$HorRat_r$	0.18	0.77	0.69	5.29

For each material, the HorRat was in the normal range of expected values for measurements in the given concentration regime. This suggests that the LVAP method is consistent, and the larger standard deviation for the APC01 may also be due to the concentration regime and not solely the material complexity. Thus, it is possible that an air-impermeable material with permeation values similar to those for APC01 may have a standard deviation more similar to APC01 than to latex.

A modification to the Horwitz calculation can be used to describe the contribution of concentration regime to the overall precision of the test method for each material. The HorRat normalizes the variance to the expected standard deviation predicted for a given concentration regime. This normalization enables a direct comparison of the method for the two material types. As part of this discussion, statistical outliers were removed to highlight the differences in standard deviation between the two material types. The updated Horwitz calculations are presented in Table 58. Here, a separate column was added to include a third day of APC01 testing, Test F, for which the preconditioning temperature requirement was not met. However, the preconditioning moisture requirement, as measured by absolute humidity, was met in this test. Additional information is provided in Section 5.4.5.

Table 58. HorRat Describes Concentration as a Source of Variability: Statistical Outliers Removed

Parameter	Spiked PTFE Samples	Latex		APC01	
Time	—	24 h	48 h	24 h	
Test days (no.)	7	5	2	2	3*
Average measured mass (µg)	5892	4814	5343	14.04	14.50
C	3.72E-04	3.06E-04	3.38E-04	8.89E-07	9.17E-07
$PRSD_R$ (%)	6.54	6.73	6.63	16.17	16.10
S_r (%)	1.2	3.2	3.6	13.8	11.9
$HorRat_r$	0.18	0.48	0.54	0.85	0.74

*Includes data from Test F, in which the preconditioning temperature requirement was not met.

The HorRat for latex was approximately half of that for APC01. This suggests that although the concentration regime was an important contributing factor with regard to variability, it was not the sole factor. Several other factors, such as material complexity, could not be quantified but were also expected to be important contributing factors.

Fully exploring this possibility would have required additional testing that was outside the scope of this V&V. However, the effect of concentration on variability may potentially affect swatch samples tested in future programs.

7.3 Effects of Sample Processing and Analytical Instrumentation on Variability

Extractions of the APC01 and the latex permeation samples required dilution and sample handling prior to analysis. The dilution procedures, the precision of the tools and equipment, and the operators' skill were additional sources of variability in the samples.

The LC-MSMS precision was not expected to greatly influence the standard deviations of the latex and APC01 results. All samples were diluted, with a target concentration in the same general region of the calibration curve. The average area counts for the 24 h latex and APC01 analytical samples were 483,000 and 475,000, respectively. The area counts for the individual material samples are provided in Tables 40 and 42.

Quantifying the variability due to sample handling and instrumentation precision was achieved by examining the spiked PTFE samples. These extracts were handled in the same manner as the swatch samples, requiring extraction, dilution, and analysis. The IPSD for the PTFE indicates that the sample handling process was highly precise, with an expected day-to-day relative standard deviation of 5.9%, as shown in Table 54. The precision was benchmarked to an expected variability due to concentration using a Horwitz calculation. The HorRat for the PTFE samples was 0.18, as shown in Table 57. This ratio shows an exemplary level of precision, with less variability than would be considered normal for this concentration range.

7.4 Quantifying Method Sensitivity to Variance Factors

7.4.1 Sensitivity to Factor Changes Using Variance Components

The variance components introduced in Section 6.4 and defined in eqs 6–8 were used as a means to quantify the relative contributions of each factor to the overall variance of the method results. Here, the overall variance of the experimental results was the total variance component and was normalized to 100%.

For example, for latex results at a 24 h contact time (outliers removed), the interpretation of the variance components estimates was that an estimated 23.7% of the observed variance in the method response was due to unexplained "random" variation within test day, whereas the remaining 76.3% of the method variance was due to the combined effects of different test days, calibrations, and contaminant vials. The total variance component is defined by

Total variance component = residual variance component + test-day variance component (11)

where the total variance component is the total variance observed in the experimental results of a given material; the residual variance component is the random error remaining after all known sources of variance are accounted for (defined as S_r^2); and the test-day variance component is the variance due to the combined effects of test day, calibration, and vial number (defined as S_L^2).

The calculated variance components are shown in Table 59 for all data and in Table 60 with the statistical outliers removed. The results are displayed graphically in Figures 46 and 47. Here, the effect of the outliers was seen, switching the source of greater variance from residual to test-day variance.

Table 59. LVAP Variance Components for Single-Laboratory Testing: All Data

Material	**Contact Time (h)**	**Residual Variance Component: Random Error (%)**	**Test-Day Variance Component: Effect of Test-Day, Calibration, and Vial No. (%)**	**Total Variance Component (%)**
PTFE control for dosing tools	n/a	4.3	95. 7	100
APC01	24	93.0	7.0	100
Latex	24	40.3	59.7	100
	48	82.5	17.5	100

n/a, not applicable.

Table 60. LVAP Variance Components for Single-Laboratory Testing: Outliers Removed

Material	**Contact Time (h)**	**Residual Variance Component: Random Error (%)**	**Test-Day Variance Component: Effect of Test-Day, Calibration, and Vial No. (%)**	**Total Variance Component (%)**
PTFE control for dosing tools	n/a	4.3	95.7	100
APC01	24	52.0	48.0	100
Latex	24	23.7	76.3	100
	48	83.0	17.0	100

n/a, not applicable.

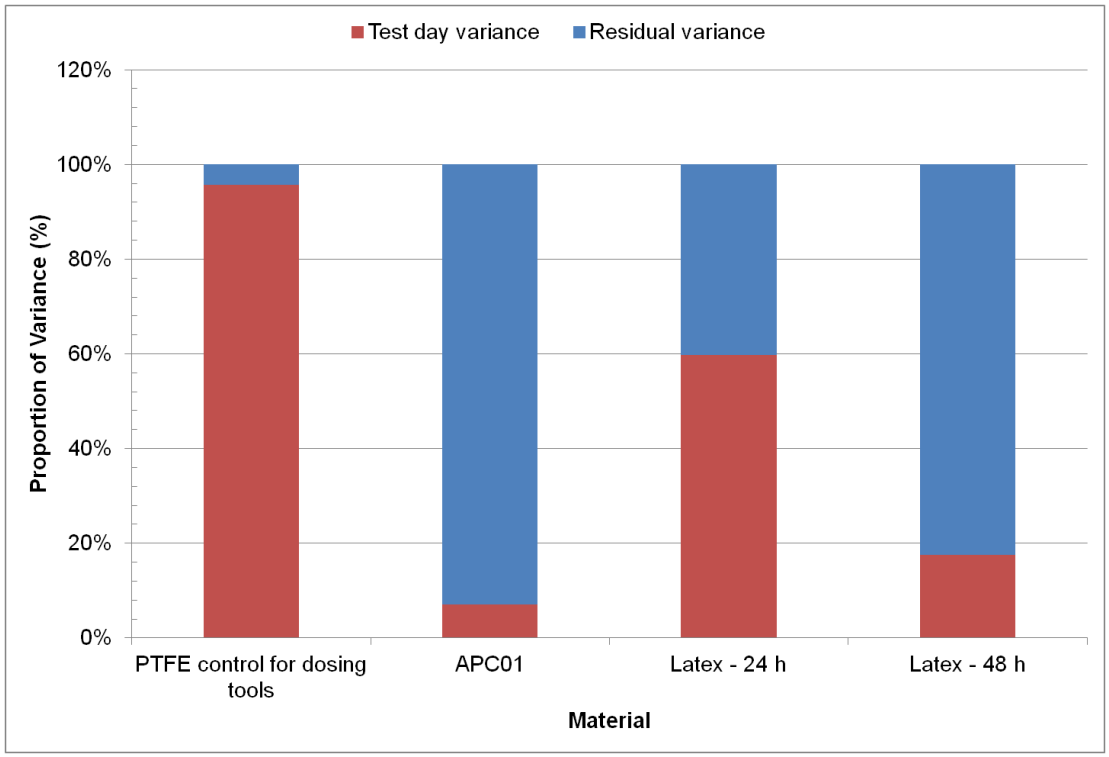


Figure 46. Stacked bar chart of variance source proportions: all data.

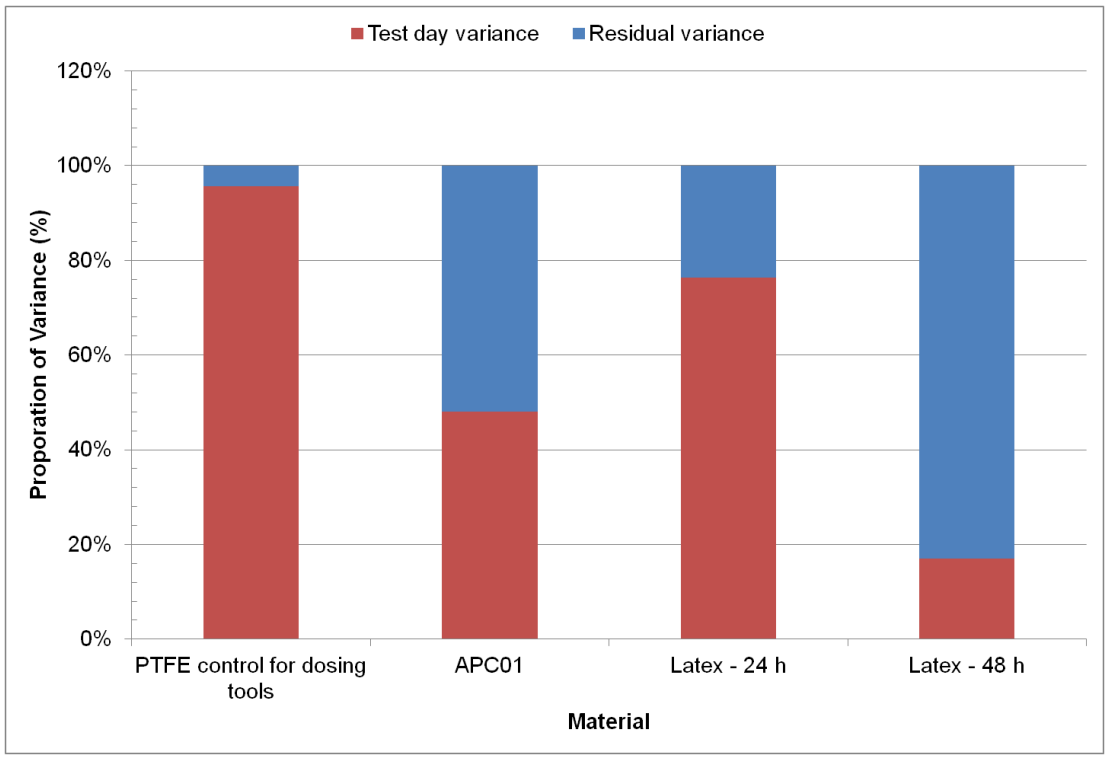


Figure 47. Stacked bar chart of variance source proportions: outliers removed.

7.4.2 Sensitivity to Changes in Concentration Regime Using Horwitz Formula

The Horwitz formula was used to quantify the sensitivity of method variability to changes in the concentration regime. This was equivalent to considering the mass-to-mass ratio (*C*) of the analyte as an additional factor. Because this was a single-laboratory study, the sensitivity of the method to changes in concentration regime was quantified using the predicted change in S_r as estimated by a modified form of eq 9, as shown by

$$\mathrm{PRSD_r}(\%) = 0.5 \times \mathrm{PRSD_R}(\%) \tag{12}$$

where $PRSD_r$ is the predicted relative repeatability standard deviation, S_r (%), expressed as a percentage of the average response of the method and generally accepted to be approximately half of the $PRSD_R$.[19]

It is generally accepted that the Horwitz historical database predicted that every reduction in concentration regime by a factor of 100 will cause the S_r to be doubled. Thus, a 2 order-of-magnitude decrease in concentration regime, in and of itself, was predicted to lead to a doubling of the "within"-test-day random error of the method results.

For example, the mass-to-mass *C* of the analyte with the APC01 material was approximately 2.4 orders of magnitude lower than the mass-to-mass *C* for the latex 24 hr material. Therefore, the Horwitz formula predicts that the S_r (%) for APC01 will be 2.4 times higher than the S_r (%) for the latex 24 h material, purely as a function of the concentration regime. The actual S_r (%) values calculated from the observed experimental data (outliers removed) for APC01 were 4.3 times higher than the observed S_r (%) for the latex 24 h material. Therefore, according to the Horwitz prediction, approximately half of the difference can be explained by the difference in concentration regime.

8. CONCLUSIONS

Through multiple years of research, LVAP has been shown to be a necessary testing component for low-volatility contaminants such as VX. The LVAP method used during the validation phase of this V&V program represents the grand total of input from multiple researchers, organizations, and stakeholders. The IPSD calculated for this method provides the necessary metrics needed to evaluate LVAP as a test method.

Additional calculations indicate that the variability may be due to both the complexity of the material type and the concentration regime of the permeation performance.

The V&V of the LVAP has been successfully completed, based on the test plan requirements and the limitations stated in this report. With the successful completion of the V&V, the LVAP method is ready for transition to the T&E community.

The V&V is only valid for the final conditions and parameters documented in this report. Additional testing may be needed for test conditions outside those detailed herein.

REFERENCES

1. *Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan*; Deputy Under Secretary of the Army for Test and Evaluation: Arlington, VA, 2010.

2. D'Onofrio, T.G. *Development of a Contact Permeation Test Fixture and Method*; ECBC-TR-1141; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2013; UNCLASSIFIED Report.

3. Stickel, G.; Andrews, A.; MacIver, B.; Steinbach, C. *Verification and Validation Test Report for Low Volatility Agent Permeation Test Method*; Customer Report to JPM P and NSRDEC; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2012.

4. *Test Operations Procedure (TOP) 8-2-501A, Permeation and Penetration of Air-Permeable, Semipermeable, and Impermeable Materials with Chemical Agents or Simulants*; TOP-8-2-501A; West Desert Test Center: Dugway Proving Ground, UT, 2013; UNCLASSIFIED Procedure.

5. *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods for Volatile Organic Chemicals*; SW-846; U.S. Environmental Protection Agency: Washington, DC, 2007.

6. *Determinative Chromatographic Separations*; Method 8000B; U.S. Environmental Protection Agency: Washington, DC, 1996.

7. *Gloves and Glove Set, Chemical Protective*; MIL-DTL-43976D; U.S. Department of Defense: Washington, DC, 2003; UNCLASSIFIED Detail.

8. *Engineering Design Handbook, Experimental Statistics*; AMC Pamphlet 706-110; Headquarters, U.S. Army Materiel Command: Washington, DC, 1969.

9. Lalain, T.; Mantooth, B.A.; Shue, M.; Pusey, S.; Wylie, D. *The Chemical Contaminant and Decontaminant Test Methodology Source Document, Second Edition*; ECBC-TR-980; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2012; UNCLASSIFIED Report.

10. Lavagnini, I.; Magno, F. A Statistical Overview on Univariate Calibration, Inverse Regression, and Detection Limits: Application to Gas Chromatography/Mass Spectrometry Technique. *Mass Spectrometry Reviews* **2007**, *26*, 1–18.

11. Mantooth, B.A.; Willis, M.; Lalain, T. *CREATIVE Decontamination System Performance Model*. Presented at the Decontamination Capability Area Process Action Team (CAPAT) Session of the Test and Evaluation Capabilities and Methodologies Integrated Process Team (TECMIPT); Arlington, VA, November 28, 2012.

12. D'Onofrio, T.G.; Ruppert, C.J.; Steinbach, C.B. *Low-Volatility Agent Permeation (LVAP) Verification Report*; Customer Report to Deputy Under Secretary of the Army for Test and Evaluation; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2014.

13. *General Requirements for the Competence of Testing and Calibration Laboratories*; ISO/IEC 17025:2005; International Organization for Standardization: Geneva, Switzerland, 2005.

14. Box, G.E.; Hunter, J.S.; Hunter, W.G. *Statistics for Experimenters: Design, Innovation, and Discovery*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, 2005.

15. Helsel, D.R. *Nondetects and Data Analysis—Statistics for Censored Environmental Data;* John Wiley & Sons: Hoboken, NJ, 2005.

16. *Accuracy (Trueness and Precision) of Measurement Method and Results—Part 3: Intermediate Measures of the Precision of a Standard Measurement Method*; 5725-3:1994(E); International Organization for Standardization: Geneva, Switzerland, 1994.

17. Montgomery, D.C. *Design and Analysis of Experiments*, 8th ed.; John Wiley & Sons: Hoboken, NJ, 2012.

18. Horwitz, W.; Kamps, L.R.; Boyer, K.W. Quality Assurance in the Analysis of Foods and Trace Constituents. *Journal of the Association of Official Analytical Chemists* **1980**, *63* (6), 1344–1354.

19. Albert, R.; Horwitz, W. A Heuristic Derivation of the Horwitz Curve. *Analytical Chemistry* **1997,** *69*(4), 789–790.

20. Horwitz, W.; Albert, R. The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision. *Journal of the AOAC International* **2006**, *89*, 1095–1109.

ACRONYMS AND ABBREVIATIONS

ANOVA	analysis of variance
APG	Aberdeen Proving Ground
ATEC	U.S. Army Test and Evaluation Command
AVLAG	Aerosol, Vapor, Liquid Assessment Group
BQL	below the quantification limit
CASARM	Chemical Agent Standard Analytical Reference Material
CBDP	Chemical and Biological Defense Program
CCV	continuing calibration verification
CHRP	Contaminated Human Remains Pouch
CoA	certificate of analysis
DART	Direct Analysis in Real Time instrument
DoD	Department of Defense
DUSA-TE	Deputy Under Secretary of the Army for Test and Evaluation
DVB	divinyl benzene
ECBC	U.S. Army Edgewood Chemical Biological Center
EE	extraction efficiency
EMS	expected mean squares
EPA	U.S. Environmental Protection Agency
HorRat	Horwitz ratio
HPLC	high-performance liquid chromatography
IEC	International Electrotechnical Commission
IP CAPAT	Individual Protection Capability Area Process Action Team
IPSD	intermediate-precision standard deviation
ISO	International Organization for Standardization
JPEO-CBD	Joint Program Executive Office for Chemical and Biological Defense
JPM NBC CA	Joint Project Manager for Nuclear, Biological and Chemical Contamination Avoidance
JPM P	Joint Project Manager for Protection
JSTO	Joint Science and Technology Office
LC	liquid chromatography
LC-MSMS	liquid chromatography-tandem mass spectrometry
LVAP	low-volatility agent permeation
MCOTEA	Marine Corps Operational Test and Evaluation Activity

MS	mass spectrometry
NIST	National Institute of Standards and Technology
NSRDEC	U.S. Army Natick Soldier Research, Development, and Engineering Center
OPTEVFOR	Operational Test and Evaluation Force
OTA	Operational Test Agency
PASB	Permeation and Analytical Solutions Branch
PTFE	polytetrafluoroethylene
QC	quality control
REML	residual maximum-likelihood method
RH	relative humidity
RSD	relative standard deviation
S&T	Science and Technology
S_L	between-test-day standard deviation
sLpm	standard liters per minute
S_r	standard deviation for measurements made under repeatability conditions
StDev	standard deviation
T&E	Test and Evaluation
TECMIPT	Test and Evaluation Capabilities and Methodologies Integrated Process Team
TOP	Test Operating Procedure
TRR	Technical Readiness Review
V&V	verification and validation
VX	*O*-ethyl *S*-[2-ethyl] methylphosphonothioate
WDTC	West Desert Test Center

APPENDIXES

These appendixes include scanned copies of the run sheets that were completed during testing and the certificate of analysis for the VX used during the verification testing.

Blank

APPENDIX A

RUN SHEETS

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
02/25/14	Dusa EE+VEA	24hrs	Steinbach/Ruppert/D'Onofrio

Permeation Rack Information		Agent Information	Equipment Serial #/Calibration Date
Hood #: 37	Rack #: Room 18	Agent Lot #: see below	Timer: S/N 2305458 xp 6/1/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: see below	Temperature Probe: S/N 122500188 xp 9/3/14
Temp Initial (°F): 3rd 90°F	Temp End (°F): 90°F	Spiking amt (mg): 50 uL dilute	Solvent amt (mL): 10 mL or 20 mL as per test plan
DVB Lot Number: 710365D, 710366D, 710362D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : ACN / 137141		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	Spike Clock Time	Aliquot Pull Time

NOTES AND COMMENTS:

COMMENTS:

Standards for spike testing

8.0 ug/mL in ACN 01272014-MVS-005
40 ug/mL in ACN 01272014-MVS-003
200 ug/mL in ACN 01272014-MVS-001

} xp 10 March 14

Test A - LVAP V&V pg 1

Location	Jar #	Sample Type	DVB Lot number	Spike Level (ug)	30 min		60 min		Dose Time	DVB Application	Extract time 30	Extract time 60
					PASB DVB	PASB Control	PASB DVB	PASB Control				
Incubator (32.2 °C)	1	Uptake	[redacted]	0.4	6480	6496	6545	6561	952	1009	1038	1108
	2	Uptake	[redacted]	0.4	6481	6497	6546	6562	953	1010	1040	1110
	3	Uptake	[redacted]	0.4	6482	6498	6547	6563	954	1012	1042	1112
	4	Uptake	[redacted]	0.4	6483	6499	6548	6564	955	1014	1044	1114
	5	Uptake	62	0.4	6484	6500	6549	6565	956	1016	1046	1116
Non-Incubator	17	Solvent spike		0.4	-	6512	-	6577	~~1048~~	1037	1103	1138
	18	Solvent spike		0.4	-	6513	-	6578	~~1049~~	1039	1110	1140
	19	Solvent spike		0.4	-	6514	-	6579	~~1050~~	1041	1111	1142
	26	Teflon immediate extract		0.4	-	6521	-	6586	956	1018	1048	1120
	27	Teflon immediate extract		0.4	-	6522	-	6587	957	1019	1051	1122
	28	Teflon immediate extract		0.4	-	6523	-	6588	958	1020	1053	1124
	35	DVB spike	[redacted]	0.4	6530	-	6595	-	9589	1000	1030	1100
	36	DVB spike	[redacted]	0.4	6531	-	6596	-	1002	1002	1032	1102
	37	DVB spike	[redacted]	0.4	6532	-	6597	-	1004	1004	1034	1104
	38	DVB spike	62	0.4	6533	-	6598	-	1006	1006	1036	1106
	39	DVB spike	[redacted]	0.4	6534	-	6599	-	1022	1022	1052	1122
	40	DVB spike	[redacted]	0.4	6535	-	6600	-	1024	1024	1054	1125
Incubator (32.2 °C)	6	Uptake	62	2	6485	6501	6550	6566	1157	1223	1254	1323
	7	Uptake	[redacted]	2	6486	6502	6551	6567	1158	1225	1255	1325
	8	Uptake	[redacted]	2	6487	6503	6552	6568	1158	1227	1257	1327
	9	Uptake	[redacted]	2	6488	6504	6553	6569	1159	1229	1259	1329
	10	Uptake	62	2	6489	6505	6554	6570	1200	1231	1301	1331

Test A - LVAP V&V pg 2

Location	Jar #	Sample Type	DVB Lot number	Spike Level (ug)	30 min PASB DVB	30 min PASB Control	60 min PASB DVB	60 min PASB Control	Dose Time	DVB Application	Extract time 30	Extract time 60
Non-Incubator	20	Solvent spike		2	-	6515	-	6580	1217	-	1247	1317
	21	Solvent spike		2	-	6516	-	6581	1219	-	1249	1319
	22	Solvent spike		2	-	6517	-	6582	1221	-	1252	1321
	29	Teflon Immediate extract		2	-	6524	-	6589	1201	1234	~~1237~~ 1306	1337 ~~1336~~
	30	Teflon Immediate extract		2	-	6525	-	6590	1201	1236	1305	1339
	31	Teflon Immediate extract		2	-	6526	-	6591	1202	1238	1310	1341
	41	DVB spike	62	2	6536	-	6601	-	1207	1207	1239	1307
	42	DVB spike	[redacted]	2	6537	-	6602	-	1209	1209	1239	1309
	43	DVB spike	[redacted]	2	6538	-	6603	-	1211	1211	1241	1311
	44	DVB spike	[redacted]	2	6539	-	6604	-	1213	1213	1243	1313
Incubator (37.2 °C)	11	Uptake	[redacted]	10	6490	6506	6555	6571	1331	1353	1423	1453
	12	Uptake	[redacted]	10	6491	6507	6556	6572	1332	1355	1425	1455
	13	Uptake	62	10	6492	6508	6557	6573	1332	~~1359~~ 1358	1428	1458
	14	Uptake	62	10	6493	6509	6558	6574	1333	1400	1430	1500
	15	Uptake	[redacted]	10	6494	6510	6559	6575	1333	1402	1432	1502
	16	Uptake - NC	[redacted]	-	6495	6511	6560	6576	—	1404	1434	1504
Non-Incubator	23	Solvent spike		10	-	6518	-	6583	1349	-	1419	1449
	24	Solvent spike		10	-	6519	-	6584	1350	-	1420	1450
	25	Solvent spike		10	-	6520	-	6585	1351	-	1421	1451
	32	Teflon Immediate extract		10	-	6527	-	6592	1334	1403	1433	1503
	33	Teflon Immediate extract		10	-	6528	-	6593	1334	1404	1434	1504
	34	Teflon Immediate extract		10	-	6529	-	6594	1335	1405	1435	1505
	45	DVB spike	[redacted]	10	6540	-	6605	-	1339		1408	1438
	46	DVB spike	62	10	6541	-	6606	-	1341		1411	1441
	47	DVB spike	62	10	6542	-	6607	-	1343		1413	1443
	48	DVB spike	[redacted]	10	6543	-	6608	-	1345		1415	1445
	49	DVB spike	[redacted]	10	6544	-	6609	-	1347		1417	1447
									1215	1215	1245	1315

50 DVB 66 2

Pot # Teflon

LVAP DATA WORKSHEET

Date: 2/25/14	Test Name: DUSA EE-VE B	Test Type/Duration: 24 h	Permeation Operators: Steinbach/Ruppert/Donofrio
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: Room 19	Agent Lot #: see below	Timer: S/N 122305458 xp 6/1/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: see below	Temperature Probe: S/N 122500188 xp 9/3/14
Temp Initial (°F): 90°F	Temp End (°F): 90°F	Spiking amt (mg): 50 ul dilute	Solvent amt (mL): 20 mL or 10 mL as per test plan
DVB Lot Number: 710362D, 710365D, 710366D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Stambach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : ACN / 137141		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

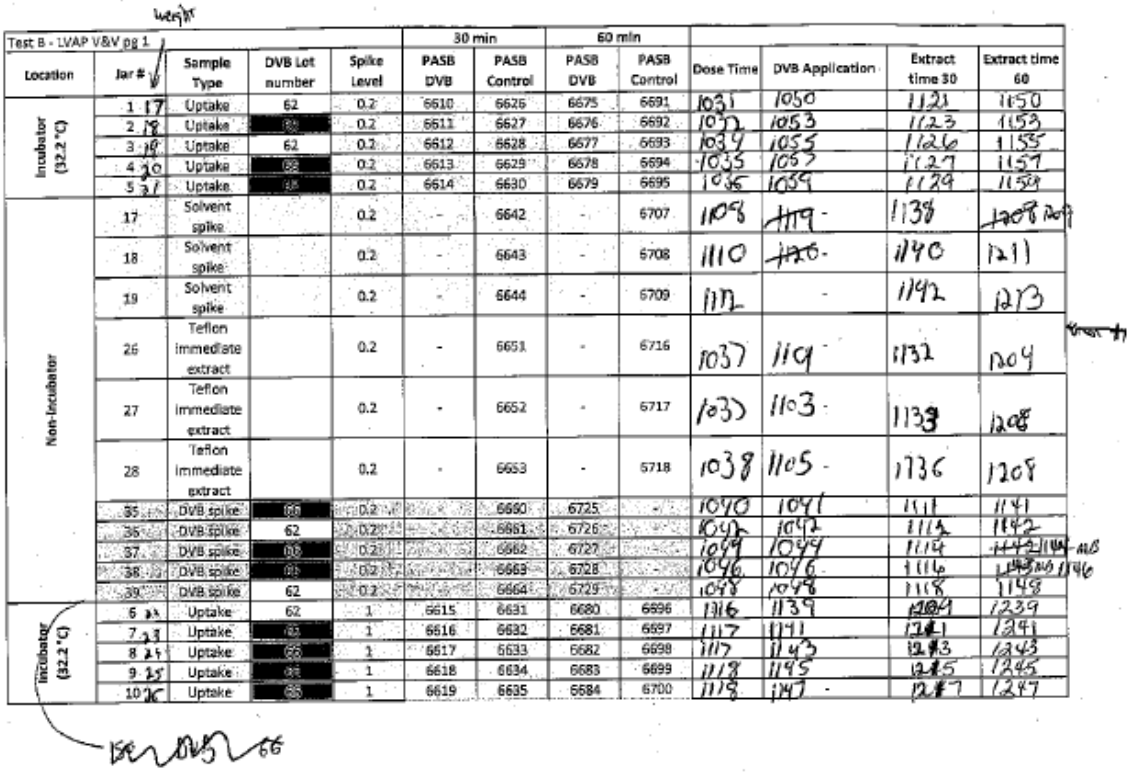
PTFE Spike (2 per trial: Beginning & End)

PTFE Sample Number	Spike Clock Time	Aliquot Pull Time

NOTES AND COMMENTS:

COMMENTS:

Standards for testing: 9.0 ug/mL VX ACN 01272014-MVS-006
20 ug/mL VX ACN 01272014-MVS-004
100 ug/mL VX ACN 01272014-MVS-002
} xp 10 March 14



Test B - LVAP V&V pg 1					30 min		60 min					
Location	Jar #	Sample Type	DVB Lot number	Spike Level	PASB DVB	PASB Control	PASB DVB	PASB Control	Dose Time	DVB Application	Extract time 30	Extract time 60
Incubator (32.2 °C)	1 17	Uptake	62	0.2	6610	6626	6675	6691	1031	1050	1121	1150
	2 18	Uptake	[redacted]	0.2	6611	6627	6676	6692	1032	1053	1123	1153
	3 19	Uptake	62	0.2	6612	6628	6677	6693	1034	1055	1126	1155
	4 20	Uptake	[redacted]	0.2	6613	6629	6678	6694	1035	1057	1127	1157
	5 21	Uptake	[redacted]	0.2	6614	6630	6679	6695	1036	1059	1129	1159
Non-Incubator	17	Solvent spike		0.2	-	6642	-	6707	1108	~~1119~~	1138	~~1208~~ 1209
	18	Solvent spike		0.2	-	6643	-	6708	1110	~~1120~~	1140	1211
	19	Solvent spike		0.2	-	6644	-	6709	1117	-	1142	1213
	26	Teflon immediate extract		0.2	-	6651	-	6716	1037	1101	1132	1204
	27	Teflon immediate extract		0.2	-	6652	-	6717	1033	1103	1133	1208
	28	Teflon immediate extract		0.2	-	6653	-	5718	1038	1105	1136	1208
	35	DVB spike	[redacted]	0.2	-	6660	6725	-	1040	1041	1111	1141
	36	DVB spike	62	0.2	-	6661	6726	-	1042	1042	1113	1142
	37	DVB spike	[redacted]	0.2	-	6662	6727	-	1044	1044	1114	~~1142~~ 1144 MB
	38	DVB spike	[redacted]	0.2	-	6663	6728	-	1046	1046	1116	~~1143~~ MB 1146
	39	DVB spike	62	0.2	-	6664	6729	-	1048	1048	1118	1148
Incubator (32.2 °C)	6 22	Uptake	62	1	6615	6631	6680	6696	1116	1139	~~1209~~	1239
	7 23	Uptake	[redacted]	1	6616	6632	6681	6697	1117	1141	1241	1241
	8 24	Uptake	[redacted]	1	6617	6633	6682	6698	1117	1143	1243	1243
	9 25	Uptake	[redacted]	1	6618	6634	6683	6699	1118	1145	1245	1245
	10 26	Uptake	[redacted]	1	6619	6635	6684	6700	1119	1147	1247	1247

Test B - LVAP V&V pg 2

Location	Jar #	Sample Type	DVB Lot number	Spike Level	30 min PASB DVB	30 min PASB Control	60 min PASB DVB	60 min PASB Control	Dose Time	DVB Application	Extract time 30	Extract time 60	
Non-Incubator	20	Solvent spike		1	-	6645	-	6710	1132	-	1204	1235	Exposure time
	21	Solvent spike		1	-	6646	-	6711	1134	-	1206	1237	
	22	Solvent spike		1	-	6647	-	6712	1137	-	1208	1239	
	29	Teflon Immediate extract		1	-	6654	-	6719	1119	1149	1219	1250	Exposure 2
	30	Teflon Immediate extract		1	-	6655	-	6720	1120	1151	1221	1252	
	31	Teflon Immediate extract		1	-	6656	-	6721	1121	1153	1224	1254	
	40	DVB spike	62	1	-	6665	6730	-	1122	1152	1152	1222	
	41	DVB spike	62	1	-	6666	6731	-	1124	1154	1154	1224	
	42	DVB spike	62	1	-	6667	6732	-	1126	1156	1156	1226	
	43	DVB spike	[redacted]	1	-	6668	6733	-	1128	1158	1158	1228	
	44	DVB spike	[redacted]	1	-	6669	6734	-	1130	1130	1200	1230	
Incubator (32.2 °C)	11 27	Uptake	[redacted]	5	6620	6636	6685	6701	1249	1312	1342	1412	
	12 28	Uptake	[redacted]	5	6621	6637	6686	6702	1249	1314	1344	1414	
	13 29	Uptake	[redacted]	5	6622	6638	6687	6703	1250	1316	1346	1416	
	14 30	Uptake	62	5	6623	6639	6688	6704	1250	1319	1349	1419	drop cSS center - new edge
	15 31	Uptake	[redacted]	5	6624	6640	6689	6705	1251	1321	1351	1421	
	16 32	Uptake - NC	[redacted]	-	6625	6641	6690	6706	—	1323	1353	1423	
Non-Incubator	23	Solvent spike		5	-	6648	-	6713	1305	~~1325~~	1335	1405	
	24	Solvent spike		5	-	6649	-	6714	1307	~~1327~~	1337	1407	
	25	Solvent spike		5	-	6650	-	6715	1309	~~1329~~	1339	1409	
	32	Teflon Immediate extract		5	-	6657	-	6722	1252	1325	1355	1425	
	33	Teflon Immediate extract		5	-	6658	-	6723	1252	1327	1357	1427	
	34	Teflon Immediate extract		5	-	6659	-	6724	1253	1328	1359	1428	
	45	DVB spike	[redacted]	5	-	6670	6735	-	1255	~~1325~~ *	1224	1357	**
	46	DVB spike	[redacted]	5	-	6671	6736	-	1257		1329	1358	***
	47	DVB spike	62	5	-	6672	6737	-	1259		1329	1359	
	48	DVB spike	[redacted]	5	-	6673	6738	-	1301		1331	1401	
	49	DVB spike	[redacted]	5	-	6674	6739	-	1303		1333	1403	

* Completed 1 min early

** Completed 2 min late

*** completed 1 min late

	35	DVB spike	66	J	1000	1030	1100
	36	DVB spike	66	J	1002	1032	1102
	37	DVB spike	66	J	1004	1034	1104
	38	DVB spike	62	J	1006	1036	1106
A	1	Uptake	66	J	1008	1038	1108
A	2	Uptake	66	J	1010	1040	1110
	3	Uptake	66	J	1012	1042	1112
	4	Uptake	66	J	1014	1044	1114
	5	Uptake	62	J	1016	1046	1116
	39	DVB spike	66	J	1022	1052	1122
	40	DVB spike	66	J	1024	1054	1124
	35	DVB spike	66	J	1041	1111	1141
	36	DVB spike	62	J	1042	1112	1142
B	37	DVB spike	66	J	1044	1114	1144
	38	DVB spike	66	J	1046	1116	1146
	39	DVB spike	62	J	1048	1118	1148
17	1	Uptake	62	J	1050	1120	1150
18	2	Uptake	66	J	1053	1123	1153
19	3	Uptake	62	J	1055	1125	1155
20	4	Uptake	66	J	1057	1127	1157
21	5	Uptake	66	J	1059	1129	1159
	40	DVB spike	62	J	1122	1152	1222
	41	DVB spike	62	J	1124	1154	1224
	42	DVB spike	62	J	1126	1156	1226
B	43	DVB spike	66	J	1128	1158	1228
	44	DVB spike	66	J	1130	1200	1230
22	6	Uptake	62	J	1139	1209	1239

23	7	Uptake	[redacted]		1141			
24	8	Uptake	[redacted]		1143			
B								
25	9	Uptake	[redacted]		1145			
26	10	Uptake	[redacted]		1147			
	41	DVB spike	62		1207			
	42	DVB spike	[redacted]		1209			
	43	DVB spike	[redacted]		1211			
A	44	DVB spike	[redacted]		1213			
	50		[redacted]		1215			
	6	Uptake	62		1223			
	7	Uptake	[redacted]		1225			
	8	Uptake	[redacted]		1227			
	9	Uptake	[redacted]		1229			
	10	Uptake	62		1231			
	45	DVB spike	[redacted]		1255			
	46	DVB spike	[redacted]		1257			
	47	DVB spike	62		1259			
	48	DVB spike	[redacted]		1301			
B	49	DVB spike	[redacted]		1303			
27	11	Uptake	[redacted]		1312			
28	12	Uptake	[redacted]		1314			
29	13	Uptake	[redacted]		1316			
30	14	Uptake	62		1319			
31	15	Uptake	[redacted]		1321			
32	16	Uptake - NC	[redacted]		1323			
	45	DVB spike	[redacted]		1338			
	46	DVB spike	62		1341			
A	47	DVB spike	62		1343			

48	DVB spike	[redacted]	✓	1345	1445	1445
49	DVB spike	[redacted]	✓	1347	1447	1447
11	Uptake	[redacted]	✓	1353	1423	1453
12	Uptake	[redacted]	✓	1355	1425	1455
13	Uptake	62	✓	1358	1428	1458
14	Uptake	62	✓	1400	1430	1500
15	Uptake	[redacted]	✓	1402	1432	1502
16	Uptake - NC	[redacted]	✓	1404	1434	1504

A

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
03/10/14	Test 1: OP. PRO.	TeSlons	Steinbach / Ruppert
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: N/A	Agent Lot #: RX-U-1223-CTF-N	Timer: SN: 122305458 exp. 06/01/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: #3 / 84-MAJ	Temperature Probe: S/N ~~122500188 exp. 09~~ CS
Temp Initial (°F): N/A	Temp End (°F): N/A	Spiking amt (mg): 6 µL	Solvent amt (mL): 20 mL
DVB Lot Number: N/A		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: A: Ruppert B: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : ACN / 137141		DVB Preparatoin (circle one): Dry Rinsed Prepped N/A	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**
N/A		
N/A		

NOTES AND COMMENTS:

COMMENTS: Initial weight: 7.99963g Final weight: 7.89391g

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
03/26/14	Dusa V&V Test D	LVAP / 24hr	Steinbach / Ruppert

Permeation Rack Information		Agent Information	Equipment Serial #/Calibration Date
Hood #: 37	Rack #: LVAP #2	Agent Lot #: VX-U-1223-GTF-1	Timer: SN: 122305458 exp. 06/01/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: 43 / 84-MAJ	Temperature Probe: S/N SN: 122500188 exp. 09/03/14
Temp Initial (°F): 90°F / 32.2°C	Temp End (°F): 90°F / 32.2°C	Spiking amt (mg): 6.0	Solvent amt (mL): Acetone / 20 mL
DVB Lot Number: 710373D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : Acetone / 136059		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)

PTFE Sample Number	Spike Clock Time	Aliquot Pull Time
PASB 7125	0820	~~0915~~ 1015
PASB 7126	0940	~~0915~~ 1015

NOTES AND COMMENTS:

COMMENTS: ~~Agent~~ Initial weight: 7.7983g
Finally weight: ~~6.555~~ 01g

Location	Jar #	Swatch type	Gasket	PASB DVB	Dose Time	Extract time	Comments
	1	Neoprene	N	7085	0821	0821	
	2	Latex	N	7086	0823	0823	
	3	Latex	Y	7087	0825	0825	Negative Control spiked
	4	Latex	N	7088	0828 0827	0827	
	5	Neoprene	N	7089	0829	0829	Negative Control
AF1	6	Butyl	Y	7090	0831	0831	
	7	Neoprene	Y	7091	0833	0833	
	8	Latex	N	7092	0835	0835	[illegible]
AB2	9	Butyl	N	7093	0837	0837	
	10	Neoprene	Y	7094	0839	0839	pic without gasket
	11	Latex	N	7095	0841	0841	
AF3	12	Butyl	N	7096	0843	0843	
	13	Latex	N	7097	0845	0845	
AB4	14	Butyl	Y	7098	0847	0847	Negative Control
BF5	15	Butyl	Y	7099	0849	0849	
	16	Neoprene	N	7100	0851	0851	photo after weight
	17	Neoprene	Y	7101	0853	0853	
BB6	18	Butyl	Y	7102	0855	0855	
	19	Latex	Y	7103	0857	0857	Negative
	20	Neoprene	Y	7104	0859	0859	
	21	Neoprene	N	7105	0858 0901	0901	
BB7	22	Butyl	N	7106	0903	0903	
	23	Latex	Y	7107	0905	0905	photo taken after o-ring removal
	24	Latex	Y	7108	0907	0907	
BF8	25	Butyl	N	7109	0909	0909	Negative Control
	26	Neoprene	N	7110	0911	0911	photo after weight
	27	Neoprene	N	7111	0913	0913	
CF9	28	Butyl	N	7112	0915	0915	
CF10	29	Butyl	Y	7113	0917	0917	
CB11	30	Butyl	Y	7114	0919	0919	
	31	Latex	N	7115	0921	0921	Negative Control
CB12	32	Butyl	N	7116	0923	0923	
DF13	33	Butyl	N	7117	0925	0925	
	34	Latex	Y	7118	0927	0927	
	35	Neoprene	Y	7119	0929	0929	
DF14	36	Butyl	Y	7120	0931	0931	
DB15	37	Butyl	Y	7121	0933	0933	
DB16	38	Butyl	N	7122	0935	0935	
	39	Latex	Y	7123	0937	0937	
	40	Neoprene	Y	7124	0939	0939	Negative Control
	Teflon			7125	0820	~~0820~~ N/A	Start Teflon
	Teflon			7126	0940	N/A	End Teflon

Room 18 Incubator (32.2°F)

LVAP DATA WORKSHEET

Date: 3/11/14	Test Name: DUSA Vav Test I	Test Type/Duration: 14 h	Permeation Operators: Steinbach & D Orazio
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: Room 18	Agent Lot #: see Below	Timer: S/N 13305488 xp 6/1/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: See below	Temperature Probe: S/N N/A
Temp Initial (°F): N/A	Temp End (°F): N/A	Spiking amt (mg): 50 ul dilute	Solvent amt (mL): 20 mL
DVB Lot Number: 710373 D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : Acetone: 136059 Methond: 102100		DVB Preparatoin (circle ~~one~~): (Dry) Rinsed (Prepped)	10 DvBs Prepped 10 DvBs Dry

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**
		N/A

NOTES AND COMMENTS:

COMMENTS:

standard for testing: 22.715 ug/ml VX in acetone
03112014-oc1

EE&UE scoping
DUSA V&V Test I

21-40

Location	Jar #	Sample Type	Solvent	Wet/Dry	PASB DVB 1st Extract	PASB DVB 2nd Extract	Dose Time	Extract time	Second extraction time
Non-Incubator	1	DVB spike	MeOH	Dry	6926	6946	~~1438~~ 1436	1436	1507
	2	DVB spike	MeOH	Dry	6927	6947	1437	1437	1508
	3	DVB spike	MeOH	Dry	6928	6948	1438	1438	1509
	4	DVB spike	MeOH	Dry	6929	6949	1439	1439	1510
	5	DVB spike	MeOH	Dry	6930	6950	1440	1440	1511
	6	DVB spike	MeOH	Wet	6931	6951	1442	1441	1512
	7	DVB spike	MeOH	Wet	6932	6952	1443	1443	1513
	8	DVB spike	MeOH	Wet	6933	6953	1444	1444	1514
	9	DVB spike	MeOH	Wet	6934	6954	1445	1445	1516
	10	DVB spike	MeOH	Wet	6935	6955	1446	1446	1517
	11	DVB spike	Acetone	Wet	6936	6956	1447	1447	1518
	12	DVB spike	Acetone	Wet	6937	6957	1448	1448	1519
	13	DVB spike	Acetone	Wet	6938	6958	1449	1449	1520
	14	DVB spike	Acetone	Wet	6939	6959	1450	1450	1521
	15	DVB spike	Acetone	Wet	6940	6960	1452	1452	1522
	16	DVB spike	Acetone	Dry	6941	6961	1453	1453	1523
	17	DVB spike	Acetone	Dry	6942	6962	1454	1454	1524
	18	DVB spike	Acetone	Dry	6943	6963	1455	1455	1525
	19	DVB spike	Acetone	Dry	6944	6964	1456	1456	1527
	20	DVB spike	Acetone	Dry	6945	6965	1457	1457	1528
	41	Solvent spike	MeOH	-	6966	-	1430	1500	-
	42	Solvent spike	MeOH	-	6967	-	1431	1501	-
	43	Solvent spike	MeOH	-	6968	-	1432	1502	-
	44	Solvent spike	Acetone	-	6969	-	1433	1503	-
	45	Solvent spike	Acetone	-	6970	-	1434	1504	-
	46	Solvent spike	Acetone	-	6971	-	1435	1505	-

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
13 March 2014	DUSA EE+UE J	24 h	Steinbach, Rupert, D'Onofrio

Permeation Rack Information		Agent Information	Equipment Serial #/Calibration Date
Hood #: 37	Rack #: Room 18	Agent Lot #: See below	Timer: S/N 122305458 xp 6/1/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: See below	Temperature Probe: S/N S/N 122500188 xp 9/3/14
Temp Initial (°F): 90°F (32.2°C)	Temp End (°F):	Spiking amt (mg): 50 uL	Solvent amt (mL): 20 mL
DVB Lot Number: 7103730		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: Acetone 136059		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	Spike Clock Time	Aliquot Pull Time

NOTES AND COMMENTS:

COMMENTS:

Standards for testing

7.27 ug/mL 03172014-003
45.43 ug/mL 03172014-002
227.15 ug/mL 03172014-001

Test J - LVAP V&V pg 1

Location	Jar #	Sample Type	Spike Level (ug)	30 min PASB DVB	30 min PASB Control	2nd extract PASB	2nd extract DVB	Dose Time	DVB Application	Extract time 30	2nd Extract time
Incubator (32.2 °C)	1	Uptake	0.4	6989	6994	7069		0819	0835	0905	0935
	2	Uptake	0.4	6990	6995	7070		0819	0838	0908	0938
	3	Uptake	0.4	6991	6996	7071		0819	0840	0910	0940
	4	Uptake	0.4	6992	6997	7072		0820	0842	0912	0942
	5	Uptake	0.4	6993	6998	7073		0820	0844	0914	0944
Non-Incubator	17	Solvent spike	0.4	-	6999	-		0824	-	0854	-
	18	Solvent spike	0.4	-	7000	-		0825	-	0855	-
	19	Solvent spike	0.4	-	7001	-		0826	-	0856	-
	26	Teflon immediate extract	0.4	-	7002	-		0821	0836	0906	-
	27	Teflon immediate extract	0.4	-	7003	-		0822	0838	0908	-
	28	Teflon immediate extract	0.4	-	7004	-		0823	0840	0910	-
	35	DVB spike	0.4	7005	-	7054		0829	-	0859	0929
	36	DVB spike	0.4	7006	-	7055		0830	-	0900	0930
	37	DVB spike	0.4	7007	-	7056		0831	-	0901	0931
	38	DVB spike	0.4	7008	-	7057		0832	-	0902	0932
	39	DVB spike	0.4	7009	-	7058		0838	-	0903	0933
Incubator (32.2 °C)	6	Uptake	2	7010	7015	7074		0848	0903	0933	1003
	7	Uptake	2	7011	7016	7075		0848	0906	0936	1006
	8	Uptake	2	7012	7017	7076		0849	0908	0938	1008
	9	Uptake	2	7013	7018	7077		0850	0910	0940	1010
	10	Uptake	2	7014	7019	7078		0851	0912	0942	1012

Test J - LVAP V&V pg 2

Location	Jar #	Sample Type	Spike Level (ug)	30 min PASB DVB	30 min PASB Control	2nd Extract PASB	2nd Extract DVB	Dose Time	DVB Application	Extract time 30	2nd Extract time
T3	20	Solvent spike	2	-	7020	-		0859	-	0929	—
	21	Solvent spike	2	-	7021	-		0900	-	0930	—
	22	Solvent spike	2	-	7022	-		0901	-	0931	—
T2 Non-Incubator	29	Teflon immediate extract	2	-	7023	-		0852	0905	0935	—
	30	Teflon immediate extract	2	-	7024	-		0853	0907	0937	—
	31	Teflon immediate extract	2	-	7025	-		0853	0909	0939	—
	40	DVB spike	2	7026	-	7059		0854	—	0924	0954
	41	DVB spike	2	7027	-	7060		0855	—	0925	0955
	42	DVB spike	2	7028	-	7061		0856	—	0926	0956
	43	DVB spike	2	7029	-	7062		0857	—	0927	0957
	44	DVB spike	2	7030	-	7063		0858	—	0928	0958
Incubator (32.2 °C)	11	Uptake	10	7031	7037	7079		0916	0932	1002	1032
	12	Uptake	10	7032	7038	7080		0917	0934	1004	1034
	13	Uptake	10	7033	7039	7081		0917	0936	1006	1036
	14	Uptake	10	7034	7040	7082		0918	0938	1008	1038
	15	Uptake	10	7035	7041	7083		0919	0940	1010	1040
	16	Uptake - NC	-	7036	7042	7084		—	0942	1012	1042
T1	23	Solvent spike	10	-	7043	-		0926	-	0956	—
	24	Solvent spike	10	-	7044	-		0927	-	0957	—
	25	Solvent spike	10	-	7045	-		0928	-	0958	—
Non-Incubator	32	Teflon immediate extract	10	-	7046	-		0919	0935	1005	—
	33	Teflon immediate extract	10	-	7047	-		0920	0937	1007	—
	34	Teflon immediate extract	10	-	7048	-		0920	0939	1009	—
	45	DVB spike	10	7049	-	7064		0921	—	0951	1021
	46	DVB spike	10	7050	-	7065		0922	—	0952	1022
	47	DVB spike	10	7051	-	7066		0923	—	0953	1023
	48	DVB spike	10	7052	-	7067		0924	—	0954	1024
	49	DVB spike	10	7053	-	7068		0925	—	0955	1025

Conflict	Time	Jar	Type	Sample	Time to next sample
	8:29	35	DVB spike	start extraction	1
	8:30	36	DVB spike	start extraction	1
	8:31	37	DVB spike	start extraction	1
	8:32	38	DVB spike	start extraction	1
	8:33	39	DVB spike	start extraction	2
	8:35	1	Uptake	start extraction	3
	8:38	2	Uptake	start extraction	2
	8:40	3	Uptake	start extraction	2
	8:42	4	Uptake	start extraction	2
	8:44	5	Uptake	start extraction	10
	8:54	40	DVB spike	start extraction	1
	8:55	41	DVB spike	start extraction	1
	8:56	42	DVB spike	start extraction	1
	8:57	43	DVB spike	start extraction	1
	8:58	44	DVB spike	start extraction	1
	8:59	35	DVB spike	1st pull	1
	9:00	36	DVB spike	1st pull	1
	9:01	37	DVB spike	1st pull	1
	9:02	38	DVB spike	1st pull	1
	9:03	39	DVB spike	1st pull	0
	9:03	6	Uptake	start extraction	2
	9:05	1	Uptake	1st pull	1
	9:06	7	Uptake	start extraction	2
TRUE	9:08	8	Uptake	start extraction	0
	9:08	2	Uptake	1st pull	2
TRUE	9:10	9	Uptake	start extraction	0
	9:10	3	Uptake	1st pull	2
TRUE	9:12	10	Uptake	start extraction	0
	9:12	4	Uptake	1st pull	2
	9:14	5	Uptake	1st pull	7
	9:21	45	DVB spike	start extraction	1
	9:22	46	DVB spike	start extraction	1
	9:23	47	DVB spike	start extraction	1
TRUE	9:24	48	DVB spike	start extraction	0
	9:24	40	DVB spike	1st pull	1
	9:25	41	DVB spike	1st pull	0
	9:25	49	DVB spike	start extraction	1
	9:26	42	DVB spike	1st pull	1
	9:27	43	DVB spike	1st pull	1
	9:28	44	DVB spike	1st pull	1
	9:29	35	DVB spike	2nd pull	1
	9:30	36	DVB spike	2nd pull	1
	9:31	37	DVB spike	2nd pull	1
TRUE	9:32	11	Uptake	start extraction	0
	9:32	38	DVB spike	2nd pull	1
	9:33	39	DVB spike	2nd pull	0

Conflict	Time	Jar	Type	Sample	Time to next sample
	9:33	6	Uptake	1st pull	1
	9:34	12	Uptake	start extraction	1
	9:35	1	Uptake	2nd pull	1
TRUE	9:36	13	Uptake	start extraction	0
	9:36	7	Uptake	1st pull	2
TRUE	9:38	14	Uptake	start extraction	0
TRUE	9:38	8	Uptake	1st pull	0
	9:38	2	Uptake	2nd pull	2
TRUE	9:40	15	Uptake	start extraction	0
TRUE	9:40	9	Uptake	1st pull	0
	9:40	3	Uptake	2nd pull	2
TRUE	9:42	16	Uptake - NC	start extraction	0
TRUE	9:42	10	Uptake	1st pull	0
	9:42	4	Uptake	2nd pull	2
	9:44	5	Uptake	2nd pull	7
	9:51	45	DVB spike	1st pull	1
	9:52	46	DVB spike	1st pull	1
	9:53	47	DVB spike	1st pull	1
TRUE	9:54	48	DVB spike	1st pull	0
	9:54	40	DVB spike	2nd pull	1
	9:55	41	DVB spike	2nd pull	0
	9:55	49	DVB spike	1st pull	1
	9:56	42	DVB spike	2nd pull	1
	9:57	43	DVB spike	2nd pull	1
	9:58	44	DVB spike	2nd pull	4
	10:02	11	Uptake	1st pull	1
	10:03	6	Uptake	2nd pull	1
	10:04	12	Uptake	1st pull	2
TRUE	10:06	13	Uptake	1st pull	0
	10:06	7	Uptake	2nd pull	2
TRUE	10:08	14	Uptake	1st pull	0
	10:08	8	Uptake	2nd pull	2
TRUE	10:10	15	Uptake	1st pull	0
	10:10	9	Uptake	2nd pull	2
TRUE	10:12	16	Uptake - NC	1st pull	0
	10:12	10	Uptake	2nd pull	9
	10:21	45	DVB spike	2nd pull	1
	10:22	46	DVB spike	2nd pull	1
	10:23	47	DVB spike	2nd pull	1
	10:24	48	DVB spike	2nd pull	1
	10:25	49	DVB spike	2nd pull	7
	10:32	11	Uptake	2nd pull	2
	10:34	12	Uptake	2nd pull	2
	10:36	13	Uptake	2nd pull	2
	10:38	14	Uptake	2nd pull	2
	10:40	15	Uptake	2nd pull	2
	10:42	16	Uptake - NC	2nd pull	

LVAP DATA WORKSHEET

Date: 04/08/14	Test Name: TSK K redo	Test Type/Duration: LVAP/24 hr	Permeation Operators: Steinbach / Ruppert
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: LVAP Chamber	Agent Lot #: VX-V-1223-CTP-V	Timer: S/N 122305498 xp 6/1/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: #13 / 84-MAJ	Temperature Probe: S/N S/N 12500188 xp 9/3/14
Temp Initial (°F): 32.2 °C	Temp End (°F): 32.1°C	Spiking amt (mg): 6 uL	Solvent amt (mL): 20 mL
DVB Lot Number: 710373D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : 134325 / Accsore		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**
7224	0914	0996
7225	1036	1110

NOTES AND COMMENTS:

COMMENTS: Initial weight: 6.55501 g Final weight: 6.33095 g

DUSA V&V Test K v2.0

Location	Jar #	Swatch type	Gasket	PASB DVB	Dose Time	Extract time	Comments
CB3	1	Butyl	Y	7184	0916	0916	
CP3	2	Butyl	Y	7185	0918	0918	
	3	Neoprene	N	7186	0920	0920	
	4	Latex	N	7187	0922	0922	
	5	Latex	N	7188	0924	0924	Negative Control
	6	Latex	Y	7189	0926	0926	
CP2	7	Butyl	N	7190	0928	0928	
	8	Latex	N	7191	0930	0930	
	9	Neoprene	N	7192	0932	0932	
	10	Latex	Y	7193	0934	0934	
	11	Neoprene	N	7194	0936	0936	
	12	Latex	Y	7195	0938	0938	
	13	Neoprene	Y	7196	0940	0940	
	14	Neoprene	Y	7197	0942	0942	~~Negative Control~~ Spiked
CB2	15	Butyl	N	7198	0944	0944	
AP1	16	Butyl	Y	7199	0946	0946	
	17	Latex	Y	7200	0948	0948	
BB1	18	Butyl	N	7201	0950	0950	
BP1	19	Butyl	N	7202	0952	0952	Negative Control
	20	Latex	Y	7203	0953	0953	
	21	Latex	N	7204	0954	0954	
AP3	22	Butyl	Y	7205	~~0954~~ 0956	0956	
BP3	23	Butyl	Y	7206	~~0955~~ 0958	0958	
BB2	24	Butyl	N	7207	1000	1000	
	25	Neoprene	N	7208	1002	1002	Negative Control
BB1	26	Butyl	N	7209	1004	1004	
BB3	27	Butyl	N	7210	1006	1006	
	28	Neoprene	Y	7211	1008	1008	Neg control
	29	Neoprene	Y	7212	1010	1010	
	30	Neoprene	N	7213	1012	1012	
	31	Latex	Y	7214	1014	1014	Negative Control
	32	Latex	N	7215	1016	1016	
	33	Neoprene	Y	7216	1018	1018	
BP2	34	Butyl	Y	7217	1020	1020	
	35	Neoprene	Y	7218	1022	1022	
AB3	36	Butyl	Y	7219	1024	1024	
AB2	37	Butyl	N	7220	1026	~~1025~~ 1026	
	38	Latex	N	7221	1028	1028	
	39	Neoprene	N	7222	1030	1030	
AP2	40	Butyl	Y	7223	1032	1032	Negative Control
	Teflon			7224	0914		End Teflon
	Teflon			7225	1036		Start Teflon

Room 18 Incubator (32.2°F)

LVAP DATA WORKSHEET

Date: 15 April 2014	Test Name: Dish viv Test L	Test Type/Duration: 48 h	Permeation Operators: Steinbach, Reppert, O'Connor
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: Room 18	Agent Lot #: see below	Timer: S/N ~~12364~~ 12305458 xp 6/1/14
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: see below	Temperature Probe: S/N S/N 12350188 xp 9/13/14
Temp Initial (°F): 32.7 °C	Temp End (°F): 32.2°C	Spiking amt (mg): 50 uL	Solvent amt (mL): 20 mL
DVB Lot Number: 7103730		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: Acetone 134325		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**

NOTES AND COMMENTS:

COMMENTS:

Same solutions as test J

03172014-003

03172014-002

03172014-001

(2)

Test 2 - LVAP V&V pg 1

Location	Jar #	Sample Type	Spike Level (ug)	30 min PASB DVB	30 min PASB Control	Dose Time	DVB Application	Extract time 30
Incubator (32.2 °C)	1	Uptake	0.4	7243	7248	1341	1421	1421
	2	Uptake	0.4	7244	7249	1341	1423	1423
	3	Uptake	0.4	7245	7250	1342	1425	1425
	4	Uptake	0.4	7246	7251	1342	1427	1427
	5	Uptake	0.4	7247	7252	1343	1429	1429
Non-Incubator	17	Solvent spike	0.4	-	7253	1345	-	1415
	18	Solvent spike	0.4	-	7254	1346	-	1416
	19	Solvent spike	0.4	-	7255	1347	-	1417
	26	Teflon immediate extract	0.4	-	7256	1343	1430	1500 1430
	27	Teflon immediate extract	0.4	-	7257	1343	1431	1501 1431
	28	Teflon immediate extract	0.4	-	7258	1344	1432	1502
	35	DVB spike	0.4	7259	-	1348	1348	1418
	36	DVB spike	0.4	7260	-	1349	1349	1419
	37	DVB spike	0.4	7261	-	1350	1350	1420
	38	DVB spike	0.4	7262	-	1351	1351	1421
	39	DVB spike	0.4	7263	-	1352	1352	1482
Non-Incubator	50	DVB Immediate Extract	0.4	7264	-	1353	Solvent 1354	1424
	51	DVB Immediate Extract	0.4	7265	-	1354	1355	1425
	52	DVB Immediate Extract	0.4	7266	-	1355	1356	1426
	53	DVB Immediate Extract	0.4	7267	-	1356	1357	1427
	54	DVB Immediate Extract	0.4	7268	-	1357	1358	1428
Incubator (32.2 °C)	6	Uptake	2	7269	7274	1402	1450	1450
	7	Uptake	2	7270	7275	1403	1452	1452
	8	Uptake	2	7271	7276	1403	1454	1454
	9	Uptake	2	7272	7277	1404	1456	1456
	10	Uptake	2	7273	7278	1404	1458	1458

Test X LVAP V&V pg 2

Location	Jar #	Sample Type	Spike Level (ug)	30 min PASB DVB	30 min PASB Control	Dose Time	DVB Application	Extract time 30
Non-Incubator #2	20	Solvent spike	2	-	7279	1407	-	1438
	21	Solvent spike	2	-	7280	1408	-	1439
	22	Solvent spike	2	-	7281	1409	-	1440
	29	Teflon	2	-	7282	1405	1500	1530
	30	Teflon	2	-	7283	1405	1501 -	1531
	31	Teflon	2	-	7284	1406	1502 1502	1532
	40	DVB spike	2	7285	-	1410	1410	1440
	41	DVB spike	2	7286	-	1411	1411	1441
	42	DVB spike	2	7287	-	1412	1412	1442
	43	DVB spike	2	7288	-	1413	1413	1443
	44	DVB spike	2	7289	-	1414	1414	1444
Non-Incubator	55	DVB Immediate Extract	2	7290	-	1416	1417	1452
	56	DVB Immediate Extract	2	7291	-	1417	1418	1453
	57	DVB Immediate Extract	2	7292	-	1418	1419	1454
	58	DVB Immediate Extract	2	7293	-	1419	1420	1455
	59	DVB Immediate Extract	2	7294	-	1420	1421	1456
Incubator (32.2 °C)	11	Uptake	10	7295	7301	1428	1508	1508
	12	Uptake	10	7296	7302	1429	1510	1510
	13	Uptake	10	7297	7303	1430	1512	1512
	14	Uptake	10	7298	7304	1431	1519	1514
	15	Uptake	10	7299	7305	1432	1516	1516
	16	Uptake - NC	-	7300	7306	—	1518	1518
#1 Creen	23	Solvent spike	10	-	7307	1437	-	1507
	24	Solvent spike	10	-	7308	1438	-	1508
	25	Solvent spike	10	-	7309	1439	-	1509
Non-Incubator	32	Teflon immediate extract	10	-	7310	1433	1509	1539
	33	Teflon immediate extract	10	-	7311	1434	1510	1540
	34	Teflon immediate extract	10	-	7312	1434	1511	1541
	45	DVB spike	10	7313	-	1440	1440	1510
	46	DVB spike	10	7314	-	1441	1441	1511
	47	DVB spike	10	7315	-	1442	1442	1512
	48	DVB spike	10	7316	-	1443	1443	1513
	49	DVB spike	10	7317	-	1444	1444	1514
#1 Non-Incubator	60	DVB Immediate Extract	10	7318	-	1445	1446	1516
	61	DVB Immediate Extract	10	7319	-	1446	1447	1517
	62	DVB Immediate Extract	10	7320	-	1447	1448	1518
	63	DVB Immediate Extract	10	7321	-	1448	1449	1519
	64	DVB Immediate Extract	10	7322	-	1449	1450	1520

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
07/09/14	Validation #1	24h / LVAP	Steinbach / Ruppert
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: LVAP Chamber	Agent Lot #: VX-U-1223-CTP-N	Timer: Fisher Sci 130755600
PreCondition RH (%): 80	Preconditioning temp (°F): 90	Agent Vial/SRC: 14 / 85-MAS	Temperature Probe: S/N Fisher Sci 125C0188
Temp Initial (°F): C 32.2	Temp End (°F): 32.2	Spiking amt (mg): 10 mg	Solvent amt (mL): 20 mL
DVB Lot Number: 710374D all samples		Pre-Conditioning Start Date/Time: 07/08/14 0800	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: 07/09/14 0800	
PTFE Extraction Solvent/Lot #: : Accton / 136059		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**
PASB 7565	0833	1035
PASB 7566	1001	1035

NOTES AND COMMENTS:

COMMENTS: Room temp. 22.8°C 57 % RH	
Sample 31 used weight 39	Initial weight: 6.474
Reloaded jars to switch	Final weight: 6.227
door left open during remodel	

Date:
DUSA V&V Test E
Validation test

Location	Jar #	Swatch type	PASB DVB	Vapor Background DVB	Dose Time	Extract time	Comments
Rogm 18 Incubator (32.2°F)	1	Latex	7525	7566	0830	0920	
	2	APC01	7526		0838	0921	
	3	Latex	7527		0840	0922	
	4	Latex - NC	7528	7567	0842	0923	Negative Control
	5	APC01	7529	7568	0844	0924	
	6	APC01	7530		0846	0926	
	7	Latex	7531		0848	0926	
	8	Latex	7532		0850	0927	
	9	APC01	7533		0852	0928	
	10	Latex	7534	7569	0854	0929	
	11	Latex	7535		0856	0930	
	12	Latex	7536		0858	0931	
	13	APC01	7537		0900	0931	
	14	Latex	7538	7570	0902	0932	
	15	Latex	7539		0904	0934	
	16	Latex	7540		0906	0936	
	17	Latex	7541	7571	0908	0938	
	18	APC01	7542		0910	0940	
	19	APC01	7543		0912	0942	
	20	Latex	7544	7572	0914	0944	
	21	Latex	7545		0916	0946	
	22	APC01	7546		0918	0948	
	23	APC01 - NC	7547	7573	0920	0950	Negative Control
	24	APC01	7548		0922	0952	
	25	APC01	7549	7574	0924	0954	
	26	Latex	7550		0926	0956	
	27	APC01	7551		0930	0958	
	28	APC01 - NC	7552	7575	0932	1000	Negative Control Spiked
	29	Latex	7553		0934	1002	
	30	APC01	7554	7576	0936	1004	N.C.
	31	APC01	7555		0938	1006	
	32	Latex	7556		0940	1008	
	33	Latex - NC	7557	7577	0942	1010	Negative Control
	34	APC01	7558		0944	1012	
	35	APC01	7559	7578	0946	1014	
	36	Latex	7560	7579	0948	1016	
	37	Latex	7561		0950	1018	
	38	Latex	7562		0952	1020	
	39	APC01	7563		0954	1022	
	40	APC01	7564	7580	0956	1024	
	Teflon		7565		0958	1035	Start Teflon
	Teflon		7566		1001	1035	End Teflon

LVAP DATA WORKSHEET

Date:	Test Name/Cloud ~~Verification #2~~	Test Type/Duration:	Permeation Operators:
07/22/14		24h LVAP	Steinbach / Ruppert
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: LVAP Chamber	Agent Lot #: VX-V-1223-CTF-N	Timer: SN: 13075560 cal. 12/16/15
PreCondition RH (%): 80%	Preconditioning temp (°F): 90	Agent Vial/SBC: 80-MAD Lot 15	Temperature Probe: S/N SN: 02501 88 xp: 9/3/14
Temp Initial (°F): C 32.2	Temp End (°F):	Spiking amt (mg): 10mg / 6uL	Solvent amt (mL): 20 mL
DVB Lot Number: 710374D		Pre-Conditioning Start Date/Time: 0800 07/22/14	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: 0800 07/23/14	
PTFE Extraction Solvent/Lot #: Acetone 117322		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**
7699	821	1022
7700	0942	1022

NOTES AND COMMENTS:

Room T = 22.1 RH = 66.05% 12.93 g/m^3 water Vaisala MP70

COMMENTS:

Date: 23 July 2014

DUSA V&V Test ~~Characterization~~ run Validation #2 - Test 24 h

Location	Jar #	Swatch type	PASB DVB	Vapor Background DVB	Dose Time	Extract time	Comments
1	1	Latex	7659		823	812	
2	2	Latex	7660		825	825	
3	3	Latex	7661	7701	827	827	
21	4	APC01	7662		829	829	
4	5	Latex - NC	7663	7702	831	831	NC
22	6	APC01	7664	7703	833	833	
23	7	APC01	7665		835	835	
5	8	Latex	7666		837	837	
6	9	Latex	7667	7704	839	839	
24	10	APC01	7668		841	841	
7	11	Latex	7669		843	843	
25	12	APC01	7670		845	845	
8	13	Latex	7671	7705	847	847	
9	14	Latex	7672		849	849	spry on ldap
~~26~~	15	~~Latex~~ APC01	7673		851	851	APC01
10	16	Latex	7674	7706	853	853	
11	17	Latex	7675		855	855	
27	18	APC01	7676	7707	857	857	
28	19	APC01 - NC	7677	7708	859	859	NC
29	20	APC01	7678		901	901	
30	21	APC01	7679	7709	903	903	
31	22	APC01	7680	7710	905	905	
12	23	Latex	7681		907	907	
13	24	Latex	7682		909	909	
32	25	APC01 - NC	7683	7711	911	911	NC
14	26	Latex	7684	7712	913	913	
33	27	APC01	7685		915	915	ring removed before post photo
34	28	APC01	7686		917	917	
35	29	APC01	7687	7713	919	919	
36	30	APC01	7688		921	921	
15	31	Latex	7689		923	923	
38	32	APC01	7690	7714	925	925	
16	33	Latex	7691		927	927	spry inside bottom
39	34	APC01	7692		929	929	
40	35	APC01	7693		931	931	
17	36	Latex	7694		933	933	
18	37	Latex	7695		930	935	
19	38	Latex - NC	7696	7715	937	937	NC
8	39	APC01	7697		939	939	
20	40	Latex	7698		941	941	
	Teflon		7699		821		Start Teflon
	Teflon		7700		942		End Teflon

Room 18 Incubator (32.2°F)

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
07/28/14	48h Validation	48h LVAP	Steinbach / Ruppert
Permeation Rack Information		**Agent Information**	**Equipment Serial #/Calibration Date**
Hood #: 37	Rack #: LVAP chamber	Agent Lot #: VX-U-1223-CTDN	Timer: SN: 13c755600 XP 12/16/15
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: #15 / 86 MAS	Temperature Probe: S/N SN: 122500188 XP 9/13/14
Temp Initial (°F): 32.2	Temp End (°F): 32.2	Spiking amt (mg): 6uL	Solvent amt (mL): 20mL
DVB Lot Number: 710376D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : Acetone		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)		
PTFE Sample Number	**Spike Clock Time**	**Aliquot Pull Time**
7756	0858	1100
7757	1022	1103

NOTES AND COMMENTS:

COMMENTS: 27 & 28 reversed (spiked negative - turned 28 into N.C.
24 25 dosing tool sprayed - seen on photos - still under PTFE
Extra 2 min between 28 & 29
Initial: 6.644g Final: 6.3473g

Validation test

Location	Jar #	Swatch type	PASB DVB	Dose Time	Extract time	Comments
Room 18 Incubator (32.2°F)	1	Latex	7716	0900	0900	
	2	Latex	7717	0902	0902	tool sprayed
	3	Latex	7718	0904	0904	
	4	Latex	7719	0906	0906	
	5	Latex - NC	7720	0908	0908	Negative Control
	6	Latex	7721	0910	0910	
	7	Latex	7722	0912	0912	
	8	Latex	7723	0914	0914	
	9	Butyl vapor	7724	0916	0916	Background vapor
	10	Latex	7725	0918	0918	
	11	Butyl vapor	7726	0920	0920	Background vapor
	12	Latex	7727	0922	0922	
	13	Latex	7728	0924	0924	
	14	Latex	7729	0926	0926	
	15	Latex	7730	0928	0928	
	16	Latex	7731	0930	0930	
	17	Latex	7732	0932	0932	
	18	Latex	7733	0934	0934	
	19	Latex	7734	0936	0936	
	20	Latex	7735	0938	0938	
	21	Latex	7736	0940	0940	
	22	Latex	7737	0942	0942	
	23	Latex	7738	0944	0944	
	24	Latex	7739	0946	0946	
	25	Latex	7740	0948	0948	tool sprayed
	26	Butyl vapor	7741	0950	0950	Background vapor
	27	Latex - NC	7742	0952	0952	~~Negative Control~~ Spiked
	28	Latex	7743	0954	0954	Negative
	29	Latex	7744	0958	0958	xtra 2 min before spike
	30	Latex	7745	1000	1000	
	31	Latex	7746	1001	1002	
	32	Latex	7747	1004	1004	
	33	Butyl vapor	7748	1006	1006	Background vapor
	34	Latex	7749	1008	1008	
	35	Butyl vapor	7750	1010	1010	Background vapor
	36	Latex	7751	1012	1012	
	37	Latex	7752	1014	1014	
	38	Latex	7753	1016	1016	
	39	Latex	7754	1018	1018	
	40	Latex	7755	1020	1020	
	Teflon	Start Teflon	7756	0858	~~+~~	Start Teflon
	Teflon	End Teflon	7757	1022		End Teflon

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
08/18/14	48h validation 2	LVAP/48h	Steinbach / Rapert

Permeation Rack Information		Agent Information	Equipment Serial #/Calibration Date
Hood #: 37	Rack #: LVAP Chamber	Agent Lot #: VX-U-1223-CTF-N	Timer: 13075560C exp-12/16/15
PreCondition RH (%): N/A	Preconditioning temp (°F): N/A	Agent Vial/SRC: 18 89-MAJ	Temperature Probe: S/N 12250058 exp-09/05/14
Temp Initial (°F): 32.2°C	Temp End (°F): 32.2°C	Spiking amt (mg): 6 µL	Solvent amt (mL): 20 mL
DVB Lot Number: 710375D		Pre-Conditioning Start Date/Time: N/A	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: N/A	
PTFE Extraction Solvent/Lot #: : Acetone		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)

PTFE Sample Number	Spike Clock Time	Aliquot Pull Time
7867	0834	1030
7868	0957	1030

NOTES AND COMMENTS:

COMMENTS:
Initial weight: 8.14084 g
Final weight: 7.88849 g

Date:
DUSA V&V Test M - 48 h
Validation test 2

Location	Jar #	Swatch type	PASB DVB	Dose Time	Extract time	Comments	
Room 18 Incubator (32.2°F)	1	Latex	7827	0836	0836		
	2	Latex	7828	0838	0838		
	3	Latex	7829	0840	0840		
	4	Latex	7830	0842	0842		
	5	Latex	7831	0844	0844		
	6	Latex	7832	0846	0846		
	7	Latex	7833	0848	0848		
	8	Latex	7834	0850	0850		
	9	Latex	7835	0852	0852		
	10	Butyl vapor	7836	0854	0854	Background vapor	
	11	Latex	7837	0856	0856		
	12	Latex	7838	0858	0858		
	13	Latex - NC	7839	~~0860~~ 0900	0900	~~Negative Control~~	Spiked
	14	Latex	7840	0902	0902	~~Background vapor~~	Neg. Control
	15	Latex	7841	0904	0904		
	16	Butyl vapor	7842	0906	0906	Background vapor	
	17	Latex	7843	0908	0908		
	18	Latex	7844	0910	0910		
	19	Butyl vapor	7845	0912	0912	Background vapor	
	20	Latex	7846	0914	0914		
	21	Latex	7847	0916	0916		
	22	Latex	7848	0918	0918		
	23	Latex	7849	0920	0920		
	24	Latex	7850	0922	0922		
	25	Latex - NC	7851	0924	0924	Negative Control	
	26	Butyl vapor	7852	0926	0926	Background vapor	
	27	Latex	7853	0928	0928		
	28	Latex	7854	0930	0930		
	29	Butyl vapor	7855	0932	0932	Background vapor	
	30	Latex	7856	0934	0934		
	31	Latex	7857	0936	0936		
	32	Latex	7858	0938	0938		
	33	Latex	7859	0940	0940		
	34	Latex	7860	0942	0942		
	35	Latex	7861	0944	0944		
	36	Latex	7862	0946	0946		
	37	Latex	7863	0948	0948		
	38	Latex - NC	7864	0950	0950	Negative Control	
	39	Latex	7865	0952	0952		
	40	Latex	7866	0954	0954		
	Teflon	Start Teflon	7867	0834	—	Start Teflon	
	Teflon	End Teflon	7868	0957	—	End Teflon	

LVAP DATA WORKSHEET

Date:	Test Name:	Test Type/Duration:	Permeation Operators:
09/16/14	Validation #2 Redo	LVAP/24h	Steinbach / Ruppert

Permeation Rack Information		Agent Information	Equipment Serial #/Calibration Date
Hood #: 32	Rack #: LVAP Chamber	Agent Lot #: VX-U-1223-CTF-N	Timer: 130755600
PreCondition RH (%): 80%	Preconditioning temp (°F): 90°F	Agent Vial/SRC: #17/88-MAJ	Temperature Probe: S/N 122500188
Temp Initial (°F): 90°F	Temp End (°F): 90°F	Spiking amt (mg): 6 mL	Solvent amt (mL): 20mL
DVB Lot Number: 710378D		Pre-Conditioning Start Date/Time: 09/15/14 0900	
Spiking Operator: Steinbach		Pre-Conditioning End Date/Time: 09/16/14 0900	
PTFE Extraction Solvent/Lot #: : Accufore / #1090		DVB Preparatoin (circle one): (Dry) Rinsed Prepped	

PTFE Spike (2 per trial: Beginning & End)

PTFE Sample Number	Spike Clock Time	Aliquot Pull Time
8228	0919	1115
8229	1042	1115

NOTES AND COMMENTS:

COMMENTS:

Date:
DUSA V&V Test N
Validation Test 2 - Redo

Location	Jar #	Swatch type	PASB DVB	Vapor Background DVB	Dose Time	Extract time	Comments
Room 18 Incubator (32.2°F)	1	APC01	8188	8230	0921		
	2	Latex	8189		0923		
	3	Latex	8190	8231	0925		
	4	APC01	8191		0927		
	5	APC01	8192	8232	0929		
	6	Latex	8193		0931		
	7	APC01	8194		0933		
	8	APC01	8195		0935		
	9	Latex - NC	8196	8233	0937		Negative
	10	APC01	8197		0939		
	11	Latex	8198	8234	0941		
	12	APC01	8199	8235	0943		
	13	Latex	8200		0945		
	14	Latex	8201		0947		
	15	Latex - NC	8202	8236	0949		Negative
	16	APC01	8203		0951		
	17	APC01 - NC	8204	8237	0953		Negative
	18	Latex	8205		0955		
	19	Latex	8206		0957		* Bad Initial Dose
	20	APC01	8207		0959		
	21	Latex	8208		0931001		
	22	APC01	8209	8238	1003		
	23	Latex	8210	8239	1005		
	24	APC01	8211		1007		
	25	APC01	8212		1009		
	26	APC01	8213		1011		
	27	Latex	8214		1013		
	28	Latex	8215		1015		
	29	Latex	8216		1017		
	30	APC01 - NC	8217	8240	1019		Negative
	31	APC01	8218		1021		
	32	Latex	8219		1023		
	33	Latex	8220	8241	1025		
	34	Latex	8221		1027		
	35	APC01	8222		1029		
	36	Latex	8223		1031		
	37	APC01	8224		1033		
	38	Latex	8225	8242	1035		
	39	APC01	8226	8243	1037		
	40	APC01	8227		1039		
	Teflon		8228		0919		Start Teflon
	Teflon		8229		1042		End Teflon

VX-U-1223-CTD-N vial 17 88-MAS
Initial = 8.2459g
Final = 7.9944g

APPENDIX B

CERTIFICATE OF ANALYSIS FOR VX

RDCB-DPC-RQ

MEMORANDUM FOR RECORD APR 0 5 2012

SUBJECT: Information on the Analysis of VX-U-1223-CTF-N

1. VX-U-1223-CTF-N is **NOT A CASARM.**

2. The following analytical data is provided for information purposes only.

   a. Oxidation-Reduction Titration (MIL-C-51105A(MU) – Analysis is traceable to NIST through 0.1 N iodine solution SRM 136e); analyzed 17 November 2011

Compound	Weight %
VX	93.4 ± 0.4
Bis Compound	0.66 ± 0.15
Free Mercaptan	0.95 ± 0.04

   b. Gas Chromatography (GC/TCD); analyzed 17-18 November 2011

	Area %
Initial Purity	93.9 ± 0.1(4)
Aggravated Storage	86.5 ± 0.0(4)

   c. GC/MSD; analyzed 17 November 2011

   VX (Area %): 96.01 ± 0.27

Compound	QM	Area %
VX, Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate	91	96.009
Diisopropylamine	91	0.273
Diethyl methylphosphonothionate, TRS	98	0.525
2-(N,N-Diisopropylamino)ethanethiol, RSH	87	0.763
Diethyl dimethylpyrophosphonate, VX pyro	95	0.249
Diisopropylaminoethyl ethyl methylphosphonate, QLO	91	0.572
Bis(diisopropylaminoethyl)disulfide, RSSR	80	0.211
Bis(S-2-diisopropylaminoethyl) methylphosphonodithiolate, Bis	91	0.352
Unidentified compounds	NA	1.046

NOTE: The Area % results represent only an approximation of the true composition due to detector saturation of the main component, VX.

ADDITIONAL ANALYTICAL INFORMATION (CONT'D)

d. ^{1}H, ^{13}C, ^{31}P NMR spectra are consistent with the following interpretation; analyzed on 17 November 2011. This method is semi-quantitative. The ratios of compounds detected in the spectra are measured. The method does not give an absolute amount of any component in the sample because no internal/external standards are used. Method reproducibility is approximately 0.5 mole % and the method detection limit is approximately 0.04 mole %.

Compound	Mole %	Weight %
O-Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX), $CH_3P(O)(OCH_2CH_3)(SR)$	93.3	94.2
Bis(S-2-[Diisopropylamino]ethyl) methylphosphonodithiolate (bis), $CH_3P(O)(SR)_2$	0.50	0.72
Diethyl dimethyldiphosphonate (VX pyro), $CH_3P(O)(OCH_2CH_3)OP(O)(CH_3)(OCH_2CH_3)$	1.04	0.91
O,O-Diethyl methylphosphonothionate (TRS), $CH_3P(S)(OCH_2CH_3)_2$	0.56	0.36
O-2-Diisopropylaminoethyl O-ethyl methylphosphonothionate (CV), $CH_3P(S)(OR)(OCH_2CH_3)$	0.13	0.13
O-(2-Diisopropylaminoethyl) methylphosphinic acid (QA), $CH_3P(O)(OR)H$	0.05	0.04
O-Ethyl methylphosphinic acid (YL), $CH_3P(O)(OCH_2CH_3)H$	(0.04)	(0.02)
Bis(2-diisopropylaminoethyl) methylphosphonate (LTO), $CH_3P(O)(OR)_2$	(0.03)	(0.04)
2-Diisopropylaminoethyl ethyl methylphosphonate (QLO), $CH_3P(O)(OR)(OCH_2CH_3)$	1.36	1.29
Diethyl methylphosphonate (TRO, DEMP), $CH_3P(O)(OCH_2CH_3)_2$	0.22	0.12
O-Ethyl methylphosphonothioic acid (EMPSH), $CH_3P(S)(OCH_2CH_3)(OH)$	0.12	0.06
Ethyl methylphosphonic acid (EMPA), $CH_3P(O)(OCH_2CH_3)(OH)$	0.24	0.11
$CH_3P(S)(OCH_2CH_3)OP(O)(CH_3)(OCH_2CH_3)$ (Unsym Pyro)	0.14	0.13
$CH_3P(S)(OCH_2CH_3)SP(S)(CH_3)(OCH_2CH_3)$ (PSP pyro)	0.05	0.05
Other pyros	0.09	0.09
2,2-bis(diisopropylamino)ethanethiol (RSH)	1.11	0.67
Other compounds δ 69-115, P=S type	0.12	0.12
Other compounds δ 37-69, R'P(O)(SR")-, $(R'O)_3P(S)$, and $R'_2P(O)$- types	0.78	0.79
Other compounds δ 19-37, phosphonic acids/esters	0.13	0.13
Other compounds δ 0-19, other acids	(0.02)	(0.02)

$R = CH_2CH_2N[CH(CH_3)_2]_2$
R' & R" are unknown
( ) denotes at or below MDL

Lot # VX-U-1223-CTF-N

ADDITIONAL ANALYTICAL INFORMATION (CONT'D)

3. This material should be stored at or below 4°C.

Suzanne A. Procell APR 0 5 2012

SUZANNE A. PROCELL
CASARM Administrator

Document No. 000005

DISTRIBUTION LIST

The following individuals and organizations received one Adobe portable document format (pdf) electronic version of this report:

U.S. Army Edgewood Chemical
Biological Center
U.S. Army RDECOM
RDCB-DRT-O
ATTN: D'Onofrio, T.
Kuperman, R.

Defense Threat Reduction Agency
DTRA/RD-CBD-T
ATTN: Ward, T.
J9-CBS
ATTN: Moore, E.

ECBC Technical Library
RDCB-DRB-BL
ATTN: Foppiano, S.
Stein, J.

Defense Technical Information Center
DTIC OA

Department of Homeland Security
DHS-ORD-CSAC
ATTN: Famini, G.

G-3 History Office
U.S. Army RDECOM
ATTN: Smart, J.

Office of the Chief Counsel
AMSRD-CC
ATTN: Upchurch, V.

ECBC Rock Island
RDCB-DE
ATTN: Lee, K.


EDGEWOOD CHEMICAL BIOLOGICAL CENTER
A U.S. Army RDECOM Laboratory