GLP STUDY REPORTS

Conducted for Pure Maintenance Holdings by Element (Eagan, MN)

2024

STUDY BACKGROUND

The Why?

Pure Maintenance sought to evaluate the effectiveness of their VaPure fogging device and process through independent third-party testing. To achieve this, Element was engaged to conduct GLP testing, which provided objective verification of the device's efficacy. This testing not only supported the claims made by Pure Maintenance regarding the device's performance but also facilitated the integration of the Pure Maintenance process into the VigorOx label. The results from this testing provided valuable evidence of the device's effectiveness in real-world applications.

What is GLP?

Good Laboratory Practice (GLP) is **a rigorous quality system that ensures scientific studies are conducted with precision, reliability, and integrity,** particularly in regulatory environments. When efficacy testing is performed in a GLP-compliant laboratory like Element, it signifies that the study followed strict protocols for data accuracy, reproducibility, and impartiality. **These standards, established by agencies such as the EPA, FDA, and OECD, guarantee that testing is conducted under controlled conditions, using validated methods and thorough documentation**. As a result, GLP-certified data carries industry-wide credibility, making it highly respected for regulatory approvals, product claims, and safety assessments. The following report details the efficacy testing conducted on both non-porous and porous surfaces, demonstrating a commitment to scientific excellence and product validation through the expertise of a trusted GLP laboratory.

Element Lab element

Element is one of the world's leading global providers of testing, inspection, and certification services for a diverse range of materials, products, and technologies. They are the premier partner for product developers, manufacturers, and users of antimicrobial pesticides and biocide products.

Element's consultative team of regulatory and scientific experts have a strong track record of more than three decades generating GLP-compliant data that is accepted by global authorities such as the U.S. Environmental Protection Agency (EPA), Health Canada, Australian Therapeutic Goods Administration (TGA), and European Chemicals Agency or individual European Member State agencies.

STUDY DESIGN

The Microorganisms

The selection of microbial test organisms is *critical* for assessing the broad-spectrum efficacy of a disinfecting system. To ensure the device's effectiveness against a variety of pathogens, a range of microbes was chosen, each representing different categories of microorganisms and infection scenarios. These include **fungi, bacteria, and mold,** which are commonly encountered in homes, healthcare and other controlled environments. The chosen organisms serve to evaluate the VaPure device's ability to address diverse threats, from ***fungal infections*** to ***antibiotic-resistant bacteria*** to ***mold spores***, ensuring the system provides comprehensive protection across different microbial challenges. They are:

- **Trichophyton interdigitale** – This genus includes dermatophytes that cause fungal infections in humans and animals, such as athlete's foot and ringworm. It's a good test organism for assessing anti-fungal and disinfectant properties.
- **Pseudomonas aeruginosa** – A common, opportunistic bacterial pathogen known for its resistance to many disinfectants and antibiotics. It's a frequent cause of hospital-acquired infections, making it a crucial test microbe for evaluating antimicrobial effectiveness.
- **Staphylococcus aureus** – A common bacterium that can cause skin infections, food poisoning, and more serious conditions like pneumonia and sepsis. Some strains (like MRSA) are highly resistant to antibiotics, making it an important challenge organism for disinfectant testing.
- **Aspergillus niger** – A mold species that produces spores and is a common contaminant in indoor environments, including air and surfaces. Testing against this fungus ensures that the fogging process is effective against airborne fungal spores, which can be problematic in homes and buildings.

STUDY DESIGN CONT...

Testing on Different Surface Types: Porous vs. Non-Porous

Testing disinfecting devices on both porous and non-porous surfaces is essential because these surfaces are commonly found in real-world environments, each presenting unique challenges. **Non-porous surfaces, such as countertops**, allow disinfectants to effectively contact and treat microbes, making them easier to disinfect. In contrast, **porous surfaces—including fabrics and carpets**—can trap microbes, limiting disinfectant penetration and making decontamination more challenging. **Evaluating both surface types ensures that the device demonstrates consistent performance across various environmental conditions, from smooth, easily cleaned surfaces to complex, absorbent materials.**

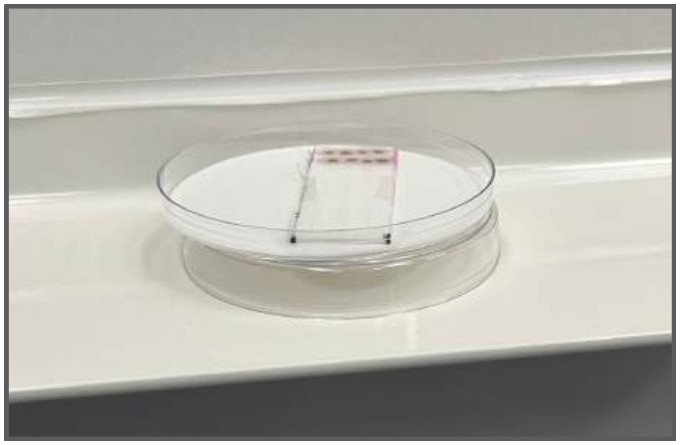
Experimental Set-up

Non-porous

Glass Microscope Slides (Non-Porous Surface)

- **Purpose**: Used as test carriers for inoculating fungal and bacterial organisms.
- **Benefits**:
  - Smooth, non-porous surface allows accurate microbial exposure.
  - Easy to observe, recover microbes, and dispose of post-testing.
  - Ensures consistent inoculation for repeatable results.
- **Procedure**: Inoculated slides exposed to the VaPure device to assess effectiveness.

Porous



Cotton Fabric (Porous Surface)

- **Preparation**: Fabric swatches are prepped by boiling in a scouring solution, rinsing, and air-drying to remove wetting agents.
- **Composite Carriers**: Fabric swatches are taped to glass slides, autoclaved, and sterilized before inoculation with the fungal organism.
- **Procedure**: Fabric-wrapped glass slides are exposed to the VaPure fogging device, simulating the challenging disinfecting conditions of porous surfaces.

STUDY SET UP

The Test Room

Description

Element invested considerable time and resources into the development of **a specialized testing chamber designed to replicate real-world environmental conditions** for evaluating devices such as foggers. Spanning **21.5' x 15' x 12'**, this advanced test room facilitates sophisticated pathogen testing. Shelving was strategically placed at varying heights and positions to assess the fumigant's efficacy in eliminating microorganisms in multiple locations. **A total of 22 testing sites were equipped with open petri dishes containing glass slides and cotton fabric carriers inoculated with bacteria, mold, and fungi**. Given the challenge presented by porous materials like cotton—requiring deep penetration of the fumigant to achieve lethality—this carefully designed setup ensured rigorous and comprehensive evaluation.

Figure 1: Sample Diagram of Carrier Placement in a Room Enclosure

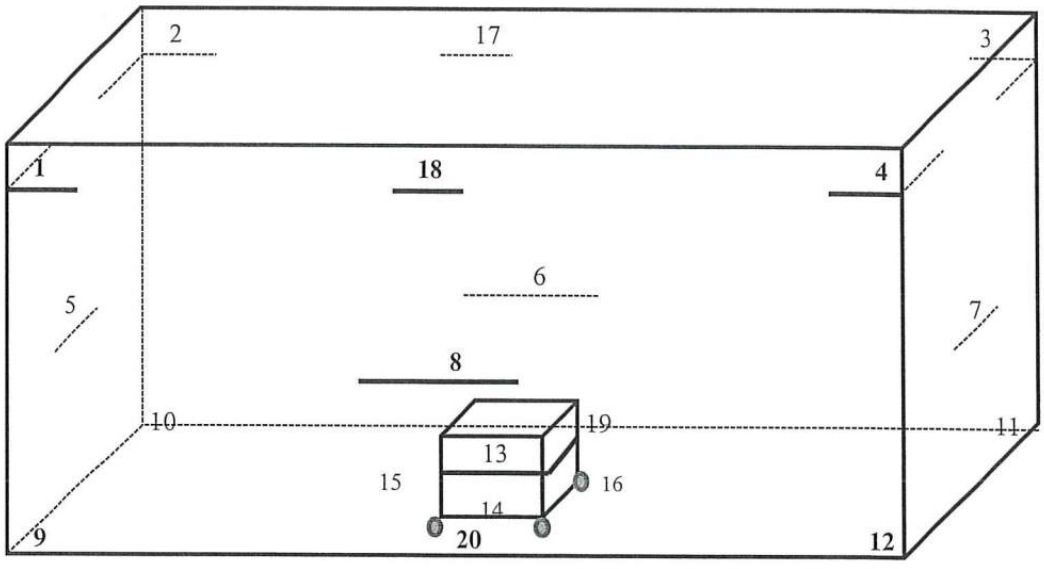


Figure 1: Illustration of the testing room is not necessarily to scale. Shelves 1-4 represent upper corners of the room. Shelves 5-8 represent central locations on all wall faces. Spaces 9-12 represent the lower corners of the room. Spaces 13 and 14 on a 3-tier laboratory cart represent spaces underneath horizontal surfaces. Spaces 15 and 16 represent central locations on or near the floor. Spaces 17 & 18 represent upper central wall faces and spaces 19 and 20 represent lower central wall faces on or near the floor.

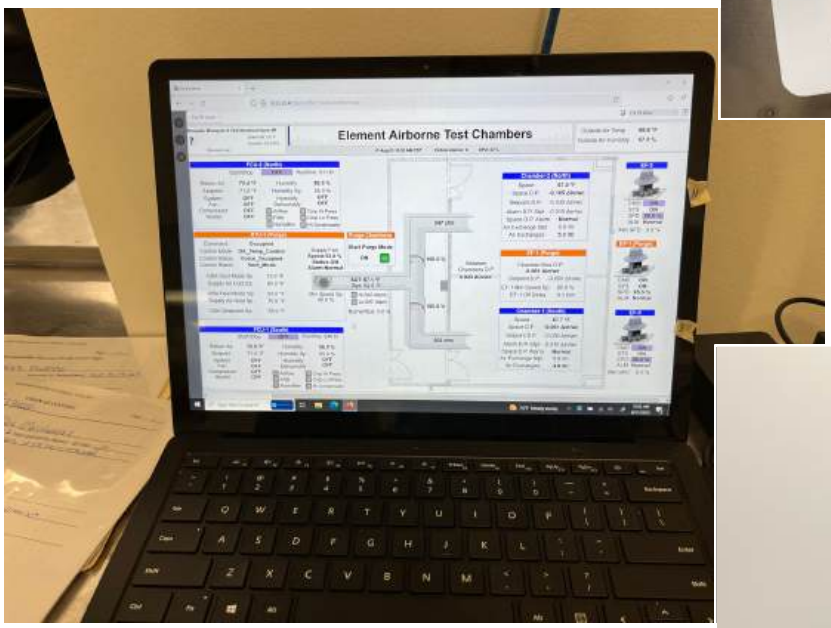
STUDY SET UP CONT...

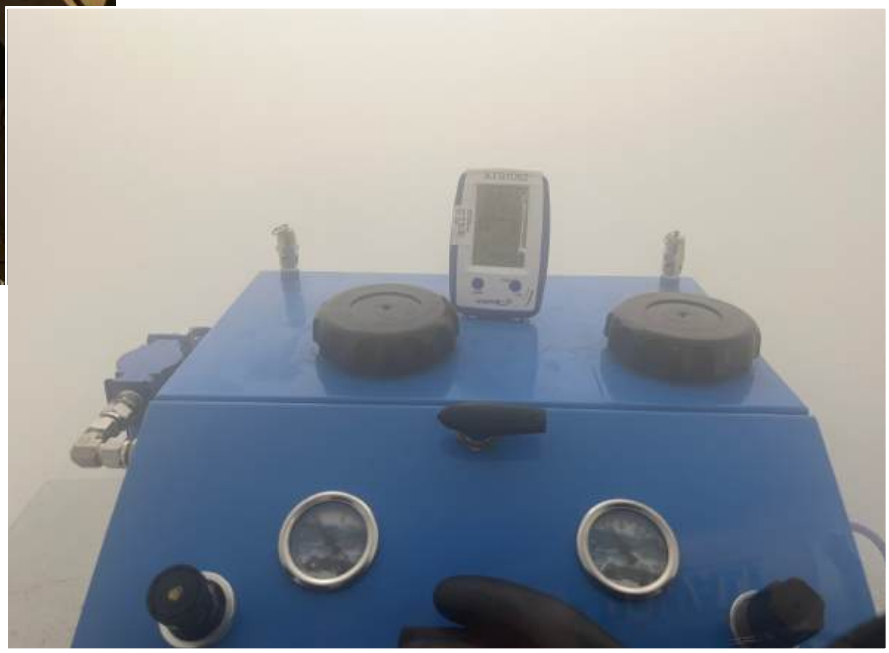
EXPERIMENTAL PROCEDURE

The Fogging Process

1. **Preparing the Fogging Device**
   - **The fogging machine** was filled with a disinfectant solution and placed in the middle of the room. The solution used in the test contained hydrogen peroxide and peroxyacetic acid—strong disinfectants designed to kill mold and bacteria.
2. **Releasing the Fog**
   - **The device was turned on for 15 minutes**, releasing a dry fog of disinfectant throughout the enclosed space. This saturated airspace and condensated on surfaces, including the glass slides covered in mold.
3. **Waiting Period (Dwell Time)**
   - **After the fogging stopped,** the room was left untouched for 45 minutes. This waiting period (called dwell time) allowed the disinfectant to fully interact with the mold on the test surfaces.
4. **Clearing the Air**
   - **Once the dwell time was over**, the room's exhaust system was turned on at full power and ran for several hours to remove the chemicals from the air until it was safe for researchers to enter.
5. **Checking the Results**
   - **After the room was cleared,** the glass slides were carefully collected and tested to see how much mold survived the fogging process.

EXP. PROCEDURE CONT...





Data Collection and Processing

After the fogging and aeration process, the lab followed the steps below to assess microbial viability and determine the efficacy of the VaPure fogging process:

01

Carrier Collection

Each inoculated glass slide was carefully retrieved from its designated location within the test chamber using sterile forceps to prevent cross-contamination.

02

Neutralization and Elution:

The slides were transferred into tubes containing a neutralizing subculture media, designed to halt any residual antimicrobial activity and allow viable microorganisms to be detected.

03

Data Analysis:

The CFU counts from treated carriers were compared against untreated controls to calculate the log reduction value (LRV), which represents the degree of microbial inactivation achieved by the fogging process.

04

Incubation:

The samples were incubated under optimal conditions for growth of each microorganism. This step ensured that any surviving microorganisms could proliferate, providing an accurate measure of the treatment's effectiveness.

05

Microbial Enumeration:

After incubation, the presence of colony-forming units (CFUs) was assessed. Any microbial growth was quantified to determine the reduction in viable spores compared to control samples. See results below.

RESULTS – Non-porous

Trichophyton interdigitale

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	*Trichophyton interdigitale* (ATCC 9533)	15 minute device run time 45 minute dwell	22	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	*Trichophyton interdigitale* (ATCC 9533)	15 minute device run time 45 minute dwell	22	0

* Number of carriers showing growth of the test organism.

Of the 22 carriers dispersed throughout the test room, **zero** showed growth of Trichophyton interdigitale.

Aspergillus niger

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	*Aspergillus niger* (ATCC 6275)	15 minute device run time 45 minute dwell	22	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	*Aspergillus niger* (ATCC 6275)	15 minute device run time 45 minute dwell	22	0

* Number of carriers showing growth of the test organism.

Of the 22 carriers dispersed throughout the test room, **zero** showed growth of Aspergillus niger.

RESULTS – Non-porous

Pseudomonas aeruginosa
& Staphylococcus aureus

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	*Pseudomonas aeruginosa* (ATCC 15442)	15 minute device run time 45 minute dwell	66	0
	Staphylococcus aureus (ATCC 6538)		66	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	*Pseudomonas aeruginosa* (ATCC 15442)	15 minute device run time 45 minute dwell	66	0
	Staphylococcus aureus (ATCC 6538)		66	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-6	*Pseudomonas aeruginosa* (ATCC 15442)	15 minute device run time 45 minute dwell	66	0
	Staphylococcus aureus (ATCC 6538)		66	0

* Number of carriers showing growth of the test organism.

Of the 66 carriers dispersed throughout the test room, **zero** showed growth of Pseudomonas aeruginosa and Staphylococcus aureus.

RESULTS – Porous

Trichophyton interdigitale

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	*Trichophyton interdigitale* (ATCC 9533)	15 minute device run time 45 minute dwell	22	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	*Trichophyton interdigitale* (ATCC 9533)	15 minute device run time 45 minute dwell	22	0

* Number of carriers showing growth of the test organism.

Of the 22 carriers dispersed throughout the test room, **zero** showed growth of Trichophyton interdigitale.

Aspergillus niger

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	*Aspergillus niger* (ATCC 6275)	15 minute device run time 45 minute dwell	22	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	*Aspergillus niger* (ATCC 6275)	15 minute device run time 45 minute dwell	22	0

* Number of carriers showing growth of the test organism.

Of the 22 carriers dispersed throughout the test room, **zero** showed growth of Aspergillus niger.

RESULTS – Porous

Pseudomonas aeruginosa & Staphylococcus aureus

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	*Pseudomonas aeruginosa* (ATCC 15442)	8 minute 50 second device run time 45 minute dwell	66	0
	Staphylococcus aureus (ATCC 6538)		66	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	*Pseudomonas aeruginosa* (ATCC 15442)	15 minute device run time 45 minute dwell	66	0
	Staphylococcus aureus (ATCC 6538)		66	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-6	*Pseudomonas aeruginosa* (ATCC 15442)	15 minute device run time 45 minute dwell	66	0
	Staphylococcus aureus (ATCC 6538)		66	0

* Number of carriers showing growth of the test organism.

Of the 66 carriers dispersed throughout the test room, **zero** showed growth of Pseudomonas aeruginosa and Staphylococcus aureus.

LOG REDUCTION
(Non-porous)

Microorganism	Lot	Control Log_{10} Value	Log Reduction
Trichophyton interdigitale	03173-4	7.11	7.11
	03173-5	7.35	7.35
			7.23 ← mean
Aspergillus niger	03173-4	6.50	6.50
	03173-5	6.15	6.15
			6.33 ← mean
Pseudomonas aeruginosa	03173-4	7.38	7.38
	03173-5	7.04	7.04
	03173-6	7.20	7.20
			7.21 ← mean
Staphylococcus aureus	03173-4	7.55	7.55
	03173-5	6.56	6.56
	03173-6	6.81	6.81
			6.97 ← mean

LOG REDUCTION
(Porous)

Microorganism	Lot	Control Log_{10} Value	Log Reduction
Trichophyton interdigitale	03173-4	5.00	5.00
	03173-5	4.93	4.93
			4.97 ← mean
Aspergillus niger	03173-4	5.15	5.15
	03173-5	4.80	4.80
			4.98 ← mean
Pseudomonas aeruginosa	03173-4	7.38	7.38
	03173-5	7.04	7.04
	03173-6	7.20	7.20
			7.21 ← mean
Staphylococcus aureus	03173-4	7.55	7.55
	03173-5	6.56	6.56
	03173-6	6.81	6.81
			6.97 ← mean

LOG REDUCTION

Understanding Log Values, Log Reduction, and the Limit of Detection

Log Values: Quantifying Microscopic Populations

Logarithmic values (or "log values") offer a simplified way to represent very large numbers—especially when dealing with microscopic organisms that are too numerous to count individually. Rather than listing every organism, scientists use powers of ten to express population size:

→ 1 organism = Log 0
→ 10 organisms = Log 1
→ 100 organisms = Log 2
→ 1,000 organisms = Log 3
→ 10,000 organisms = Log 4
→ 100,000 organisms = Log 5
→ 1,000,000 organisms = Log 6
→ 10,000,000 organisms = Log 7

Key Point: Log values convey the magnitude of microbial presence.

Log Reduction: Gauging Effectiveness of Elimination

Log reduction is a metric used to evaluate how effectively a treatment reduces the number of organisms. Each log reduction corresponds to a tenfold (or 90%) decrease in the original population:

Starting with 10,000,000 organisms (Log 7):
- 1-log reduction → 1,000,000 remain (90% eliminated)
- 2-log reduction → 100,000 remain (99% eliminated)
- 3-log reduction → 10,000 remain (99.9% eliminated)
- 7-log reduction → 1 remains (99.99999% eliminated)

Key Point: Log reduction quantifies how many organisms were successfully removed or destroyed.

LOG REDUCTION (Cont.)

Limit of Detection: The Threshold of Visibility

The limit of detection (LOD) refers to the smallest number of organisms that can still be reliably measured by a test. It's akin to the lowest magnification at which something can still be seen clearly.

Key Point:
→ If a test shows “no organisms detected,” it doesn’t necessarily mean none are present—it means the number falls below the threshold of detection.

Why This Matters
→ It indicates when a treatment has reduced the microbial load to undetectable levels.
→ It provides a scientific benchmark for stating that a small amount of microbes may remain, but current tools cannot detect them.

Application in This Study

→ **Log Value (Control Log_{10} Value)**: The starting number of CFU (Colony Forming Units) ranged from ~63,000 (4.80) to ~35,000,000 (7.55) depending on the microorganism and surface type.
→ **Log Reduction:** The range of log reductions is 4.80 to 7.55. In every case, the log reduction is equal to the Log Value because all of the fungal and bacterial matter were eliminated down to the LOD.
→ **Limit of Detection:** The LOD was 20 CFU for each microorganism.

More Information About Health Care

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THANK YOU !

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