Abstract
For over two decades, I have worked at the intersection of biomedical engineering, thermal sterilization science, and autoclave systems development. During this time, I have observed the persistence of a flawed protocol in many biosafety laboratories: the addition of water to sealed biohazard bags under the belief that it enhances autoclave decontamination. This method contradicts well-established thermodynamic principles and fails under scrutiny when tested against modern standards of steam penetration and sterilization consistency. This white paper presents empirical evidence and engineering analysis demonstrating why this method is ineffective and potentially hazardous, and why it must be replaced by systems engineered for true sterilization control.

Introduction

As one of the senior biomedical engineers specializing in autoclave systems, I have had the opportunity to evaluate sterilization practices in some of the most advanced—and in some cases, outdated—biosafety environments across the country. One recurring practice that demands immediate re-evaluation is the use of water inside sealed biohazard bags during autoclave cycles, particularly Gravity and Liquid programs. Despite the wide acceptance of this method, there is a fundamental flaw: water in a sealed, insulated volume behaves as a thermal buffer—not a steam generator. My aim here is to correct the misconceptions and present results from controlled testing that underscore the physical limits of this approach.

Materials and Methods

All testing was performed using industry-standard equipment:

- **Autoclave:** Steris V-120 Scientific Autoclave
- **Indicators:** Propper Steam/Temperature Strips (Model 26410100) and McKesson Type “A” Biological Indicators (Model 73-SCS100)
- **Test Packs:** 3M Bowie-Dick Test Pack (outer wrap removed)
- **Containment Systems:** 2 mil 20-gallon autoclave bags and Safe-Decon Bio-Pro 300 with automatic pressure-balancing valves
- **Simulated Waste Load:** Hard plastic boards and light lab trash

- **Fluid Load:** 100 ml & 250 ml of warm tap water, where applicable

Three sterilization cycles were used:

1. **Pre-Vacuum (Pre-Vac):** 3-Minute purge, 4-pulse vacuum, 40-minute 121°C/250°F, 5-minute dry
2. **Gravity:** Standard purge, 40-minute 121°C/250°F, 5-minute dry
3. **Liquid:** 3 Minute purge, 40-minute 121°C/250°F
4. **Pre-Vacuum (Pre-Vav)** 3-minute purge, 4 pulse vacuum, 60-minute 121°C/250°F, 5-minute dry
5. **Gravity:** Standard purge, 60-minute 121°C/250°F, 5-minute dry

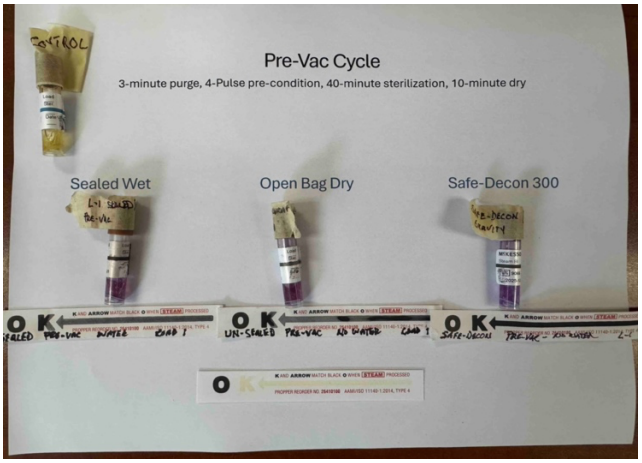
Each test condition included three sample groups:

- Sealed autoclave bag with 100 ml & 250 ml water
- Open autoclave bag without water
- Sealed autoclave bag without water
- Safe-Decon Bio-Pro 300 container (sealed, no water)

Results and Discussion

Cycle 1: Pre-Vac, No Trash, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 100ml	Neg	Yes	Bag unsealed
Open Bag/no water	Neg	Yes	
Safe-Decon Bio-Pro 300	Neg	Yes	

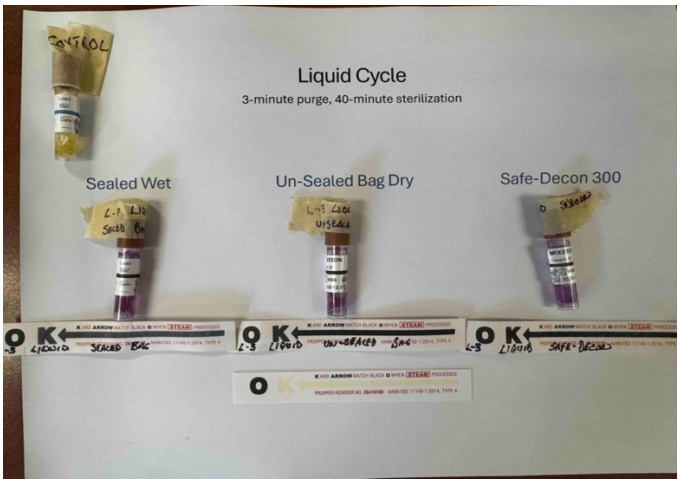


Cycle 2: Gravity/ No Trash, 40 min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 100ml	Neg	Yes	Bag unsealed
Open Bag/no water	Neg	Yes	
Safe-Decon Bio-Pro 300	Neg	Yes	

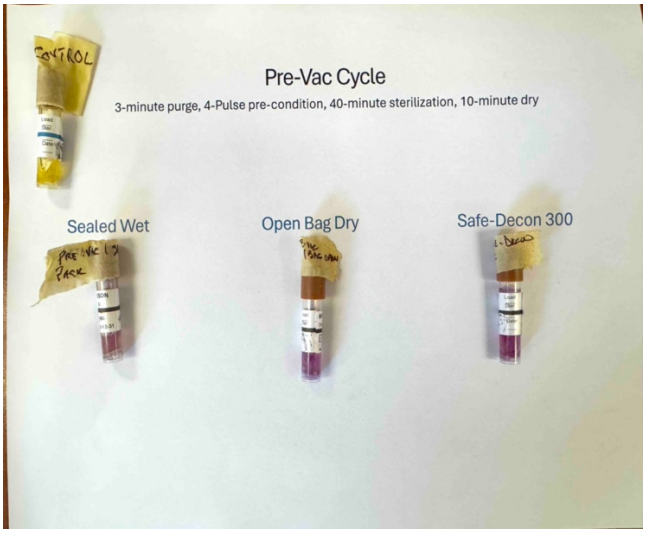
Cycle 3: Liquid, 40 Min Sterilization

Container	B.I. Indicator	Steam Indicator	Observations
Sealed Bag/water 100ml	Neg	Yes	Bag ruptured
Open Bag/no water	Neg	Yes	
Safe-Decon Bio-Pro 300	Neg	Yes	



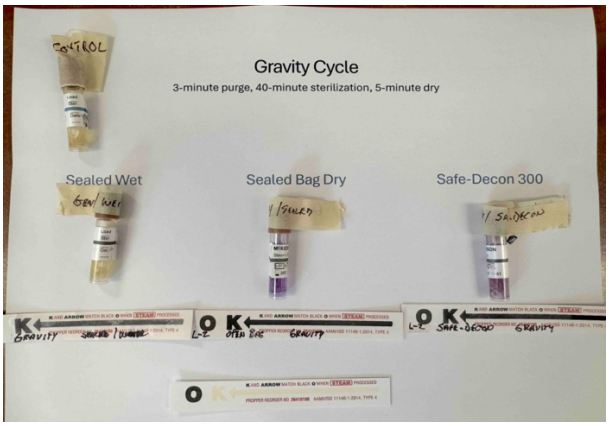
Cycle 4: Pre-Vac with Trash, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 100ml	Neg	Yes	Bag ruptured
Sealed Bag/no water	Neg	Yes	Bag ruptured
Safe-Decon Bio-Pro 300	Neg	Yes	



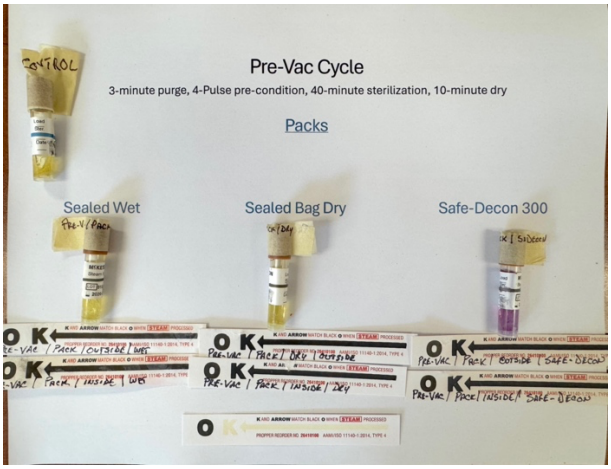
Cycle 5: Gravity with Trash, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 100ml	Pos	Yes	Bag ruptured
Sealed Bag/no water	Neg	Yes	Bag ruptured
Safe-Decon Bio-Pro 300	Neg	Yes	



Cycle 6: Pre-Vac with Trash & Sealed Pack, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 250ml	Pos	Yes	Bag Intact
Sealed Bag/no water	Pos	Yes	Bag ruptured
Safe-Decon Bio-Pro 300	Neg	Yes	

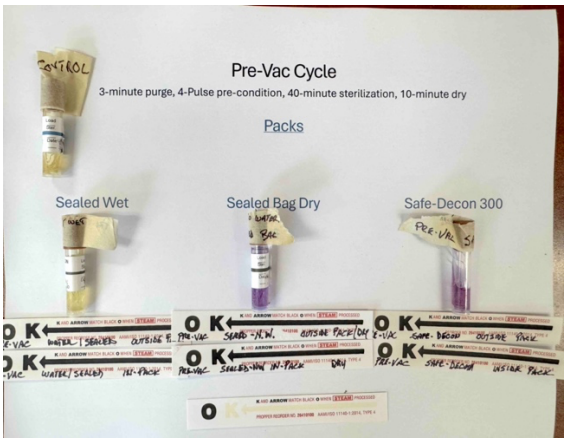


Cycle 7: Gravity with Trash & Sealed Pack, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 250ml	Pos	Yes	Bag ruptured
Closed Bag/no water	Pos	Yes	Bag Intact
Safe-Decon Bio-Pro 300	Pos	Yes	

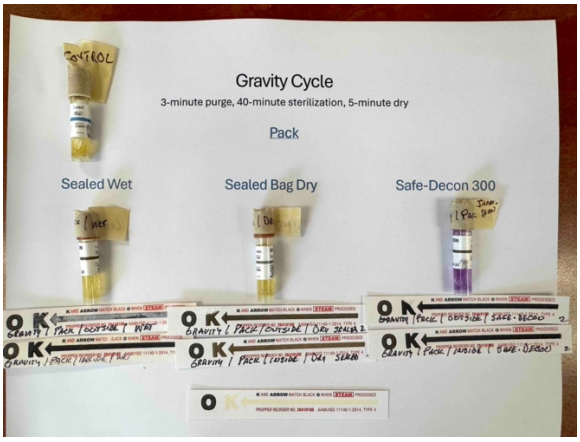
Cycle 8: Pre-Vac with Trash & Pack, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 250ml	Pos	Yes	Bag Intact
Sealed Bag/no water	Neg	Yes	Bag ruptured
Safe-Decon Bio-Pro 300	Neg	Yes	



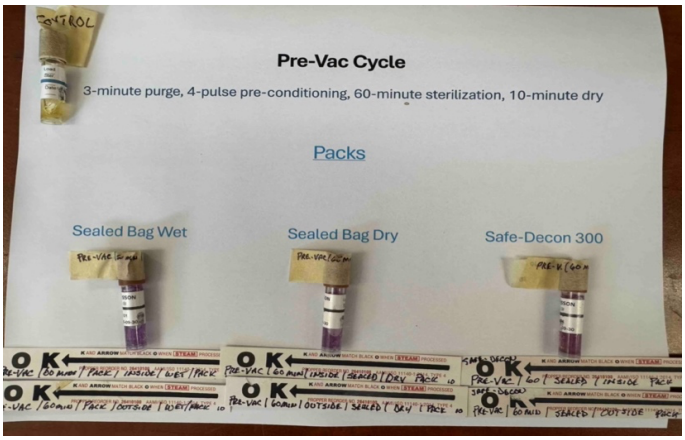
Cycle 9: Gravity with Trash & Pack, 40 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 250ml	Pos	Yes	Bag Intact
Sealed Bag/no water	Pos	Yes	Bag Intact
Safe-Decon Bio-Pro 300	Neg	Yes	



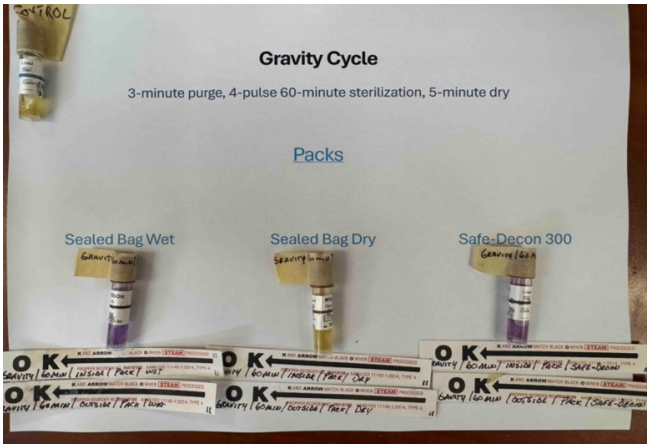
Cycle 10: Pre-Vac with Trash & Pack, 60 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 250ml	Neg	Yes	Bag ruptured
Sealed Bag/no water	Neg	Yes	Bag ruptured
Safe-Decon Bio-Pro 300	Neg	Yes	



Cycle 11: Gravity with Trash & Pack, 60 Min Sterilization

Container	*B.I. Indicator*	*Steam Indicator*	*Observations*
Sealed Bag/water 250ml	Neg	Yes	Bag ruptured
Sealed Bag/no water	Pos	Yes	Bag Intact
Safe-Decon Bio-Pro 300	Neg	Yes	



In field simulations with light waste loads:

- **Sealed bags frequently ruptured** due to internal pressure buildup
- **Steam penetration only improved after rupture**, defeating the concept of sealed containment
- **Bowie-Dick test packs inside intact water-containing bags failed**, returning **positive biological indicator**

Engineering Observations:

- Water in a sealed container insulates against heat transfer rather than promoting it
- Effective steam sterilization requires **air displacement and controlled condensation**—both of which are obstructed in sealed bags with water
- The method's success is often falsely attributed to steam produced **after a rupture**, a dangerous variable

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Physics of Sterilization Failure

Let us be technically clear: sterilization depends on the transfer of latent heat from condensing steam at a known pressure and temperature. Inserting free liquid into a sealed space introduces three problems:

- **Thermal Lag:** Water absorbs heat and delays internal temperature rise
- **No Effective Air Displacement:** In sealed bags, steam cannot circulate or replace trapped air, undermining even exposure
- **Flash Boiling Misconception:** While pressure drop may cause boiling, the resultant steam is low-pressure and insufficient for reliable sterilization

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Cycle Performance Breakdown

- **Pre-Vac:** The most effective at air removal and pressure cycling; still, sealed water-containing bags only worked **after rupture**
- **Gravity:** Insufficient steam saturation early in the cycle. Success depended on whether the bag burst
- **Liquid:** Specifically designed **not to produce steam** during its cycle. It is the least appropriate option for solid waste

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Conclusion: Autoclave Cycle Testing with Biohazard Bags

After running 9 autoclave cycles with increasing steam/heat penetration challenges, the results consistently showed repeated failures. The presence of water inside biohazard bags was found to potentially cause unintentional exposures. The observations and findings are summarized below:

- **Insulating Effect of Water:** The addition of water inside biohazard bags creates an insulating layer, keeping the bag's interior cooler since water takes longer to reach sterilization temperatures. Excessive humidity from added water can prevent adequate steam penetration. When steam contacts a cooler surface (below boiling point), it condenses, failing to deliver the required thermal energy deeper into the waste.
- **Bag Rupture Risk:** Testing showed that the presence of water inside the bags often caused them to burst, potentially leading to exposure risks. This likely occurs post-sterilization: at 15 psi, water boils at 122°C/252°F. If the pressure drops while the temperature remains high, water rapidly boils, causing sudden bag expansion and rupture. While steam indicator strips confirmed steam presence, biological indicators were positive, indicating inadequate heat penetration.
- **Importance of Proper Cycle Selection:** Selecting the correct autoclave cycle is critical for effective decontamination and exposure prevention:
    - **Liquid Cycle:** Should be a last resort for waste processing. It features a slower pressure/temperature increase and an equally slow depressurization, preventing the rapid boiling of liquids and thus minimizing steam production. This cycle may not achieve effective sterilization for biohazard waste.
    - **Gravity Cycle:** Similar to the liquid cycle, but releases steam pressure immediately after sterilization. With liquid under pressure, steam production is delayed until post-cycle depressurization. Steam indicators may show presence, but lack of sustained pressure can result in inadequate heat penetration. Bag rupture and exposure risk is significant.
    - **Pre-Vacuum (Pre-Vac) Cycle:** Recommended as the preferred method. This cycle includes a pre-conditioning phase that forces steam under pressure into the chamber and then pulls a vacuum. This ensures deeper and more effective steam and

heat penetration, reducing biological contamination risks and minimizing potential for bag rupture.

These findings highlight the dangers of using inappropriate cycles and the presence of liquids in biohazard waste bags. The Pre-Vac cycle provides the most reliable results for safe and effective sterilization.

Call to Action

As professionals responsible for laboratory safety and compliance, we must retire outdated practices that have outlived their scientific validity. S.O.P.s involving added water in sealed containers must be formally re-evaluated and replaced with validated systems designed to respect the laws of physics. Autoclave sterilization must be consistent, complete, and containable—and our protocols must reflect that standard.