Performance Quality Validation of Autoclave Vacuum, Liquid and Gravity Displacement Cycles for Sterilization of Biohazardous Waste

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INTRODUCTION

- The development of a standardized autoclave validation procedure guarantees the reliable sterilization of complex biohazardous waste (BHW) generated in public health laboratories in compliance with international biosafety standards.
- This study assesses the precise functions, strengths, and limitations of autoclave pre-vacuum, liquid, and gravity displacement cycles, validating appropriate cycles for routine sterilization.
- Results support the optimization of cycle parameters and model waste loads to achieve reliable sterilization across a diverse range of wastes and cycles.

Objectives

Design representative waste models to reflect real BHW across multiple labs.

Simulate default lab protocols with model waste and collect data.

Identify factors impacting reliability: Operational quality, waste contents, volume, time, heat and steam penetration.

Recommend evidence-based process improvements aligned with established protocols.

Standardize validation procedures for future use.

Autoclave Quality Controls

Operational Quality (OQ)

- Reliable steam/pressure; Routine QA maintenance.
- Monitoring with chemical integrators and steam/pressure quality checks.

Performance Quality (PQ)

- Reliable sterilization of biohazardous waste.
- Sterility assurance level (SAL) of 10^{-6} (1.0 CFU / 1,000,000 cycles).
- Compliant with regulatory benchmarks.

Bearss et al. 2017

Table 1. Autoclave Cycle Types

Cycle Type	Purpose	Sterilization	Feature
Liquid	General-Purpose, Liquid media.	Slow increase in steam, heat and pressure to prevent boiling.	Slow Exhaust: Slow pressure release to prevent flash boiling.
Gravity	General-Purpose, Reusable instruments.	High-velocity steam injections force chamber air out the drain.	Drying: Chamber air evacuated for a set time.
Vacuum	Dry / wrapped waste; Sensitive instruments.	Strong vacuum pulses remove air, followed by steam pressure injections.	Drying: Chamber air evacuated for a set time.

Table 2. SHL DCD Autoclave Models

Autoclave Model	Brand	Cycle Options	Vacuum Capability
N936675-01 X1	CSS	• Vacuum • Gravity • Liquid	Venturi + Liquid-Ring Pump (≤30inHg)
SSR-3A-ADVPRO	CSS	• Gravity • Liquid	Venturi (10-15inHg)
4640OD/2140DD	Easter Services	• Liquid	N/A

METHODS

Designing Representative Waste

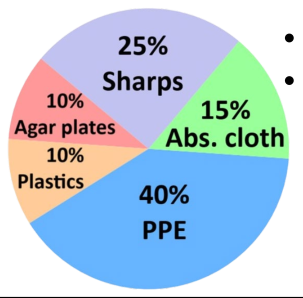
- Real biohazardous waste (BHW) cannot safely be tested for sterility. ***Representative waste*** is designed to simulate BHW.
- Reliable sterilization depends on accurately predicting waste composition, which varies by laboratory type.
- Lab experts are surveyed to assist with designing accurate models for validation.

Le et al. (2005)

Table 3. Biohazard Waste Compositions

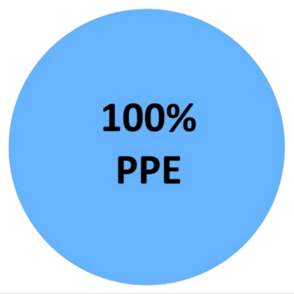
Laboratory Response Network (LRN)

LRN-M1 (*Standard Waste*)



- 4lbs
- Sharps containers were buried beneath dry PPE and cloth

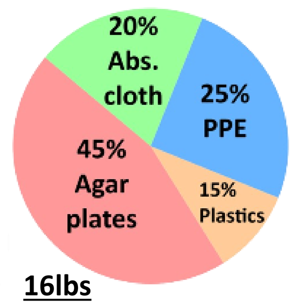
LRN-M2 (*BSL-3 PPE*)



- 2lbs
- Gowns
- Gloves
- Ankle guards
- Face shields

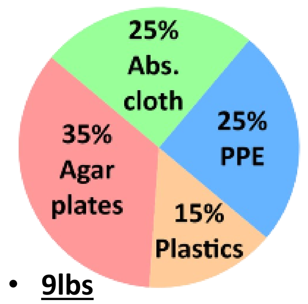
Reference Bacteriology (RB)

RB-M1 (*Primary Benchwork Waste*)



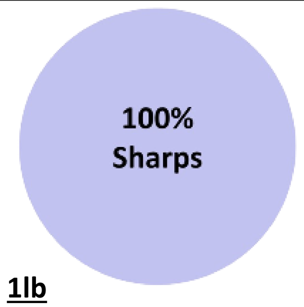
- 16lbs
- 250 agar plates

RB-M2 (*BSC Waste*)



- 9lbs
- 120 agar plates

RB-M3 (*BSC Sharps*)



- 1lb

Model Bag and Chamber Configuration

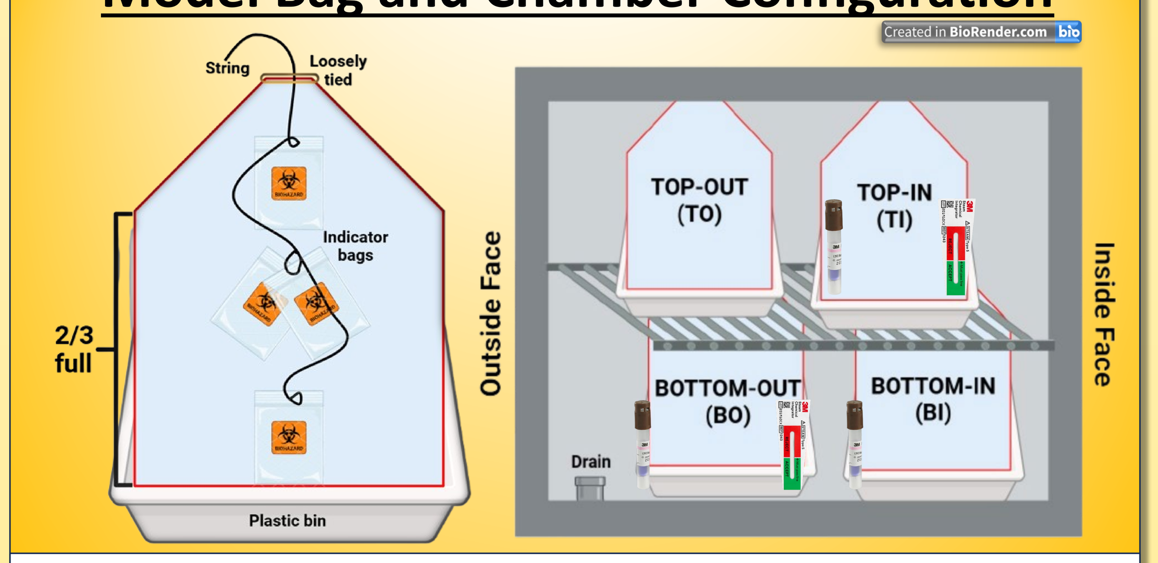
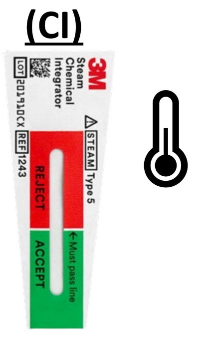


Figure 1. Indicator Bag Arrangement and Chamber Load Labelling
Technical specifications between bag and autoclave models vary between laboratories
Bags were loosely tied pre-sterilization to facilitate steam penetration

Table 4. Sterilization Indicators

Indicator Type	Methods & Features	Significance
Chemical (CI) 	• Molten pellet migrates past the accept line. • Mimics BI response but non-confirmatory.	• First validation step. • Rapid assessment of sterility before BI confirmation.
Biological (BI) 	• Fluorescently tagged, heat-resistant bacterial spores (10^6 CFU/mL). • Auto-reader detects growth in 3 hours; pH/color change confirms after 48 hours.	• Confirmatory Indicator • Validates sterilization to a Sterility Assurance Level (SAL) of 10^6. Required for final validation.

RESULTS

Triplicate Validation Standard

All indicators in a cycle must pass.

A cycle setting and load model must be successfully run in triplicate to be validated.

A cycle abortion or indicator failure during the triplicate resets the validation.

Successful triplication confirms PQ validation for designated cycle setting and bag model.

All cycles are documented for compliance tracking.

Bearss et al. 2017

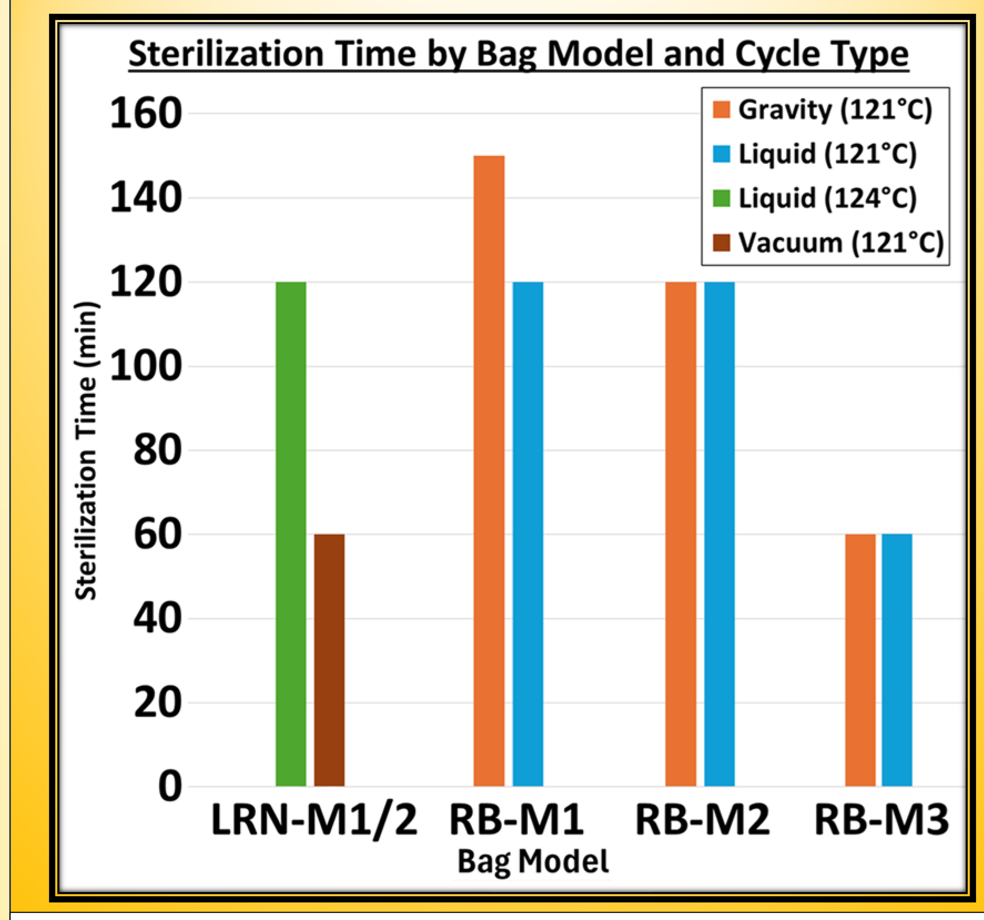


Figure 2. Validated Sterilization Times by Bag Model and Cycle Type
*LRN-M1/2, Liquid 124C used 500mL H2O in bags

Optimizing Sterilization Time to Waste Mass

- 121°C Liquid cycle mass threshold tests were performed with modified RB-M1 bags containing 250 (16lbs), 120 (9lbs), and 50 (3lbs) agar plates. Agar plates are the primary waste mass in these loads.
- 60 minutes failed to sterilize 50-plate bags.
- 90 minutes for 50-plate bags is still under evaluation:
  - Preliminary results show potential failures.
- 120 minutes was required for both 120-plate (9lbs) and 250-plate (16lbs) bag sterilization.
- Data from 120-minute tests may suggest a plateau in time-per-weight optimization.
  - *Bearss et al. (2017)* proposes that chamber volume—not load mass—is the true upper limit to sterilization capacity.
- A 150-minute Gravity cycle was validated for 250-plate bags, compared to the validated 120-minute Gravity cycle for the 120-plate bags—potentially attributed with the liquid cycle "slow-exhaust" phase—demonstrating the need for extended times with less efficient cycles.

Significance of The Drying Cycle

Drying Cycles Are Not Part of Sterilization

- Post-sterilization, in Vacuum and Gravity cycles, the vacuum evaporates condensation in the chamber.
- During drying, the chamber rapidly cools below sterilization conditions (e.g., <121°C, <15 psi). No new steam is injected.
- Useful for handling and packaging—but not sterilization.

DISCUSSION

Extreme Challenge Conditions

CHALLENGES

Steam Penetration

- Sharps containers.
- Densely-packed dry waste.
- Sealed bags within bags.

Bearss et al. (2017)

Heat Distribution

- Liquid/Gravity cycles need more time to sterilize dry lab waste.
- Waste microclimates inhibit even heating.

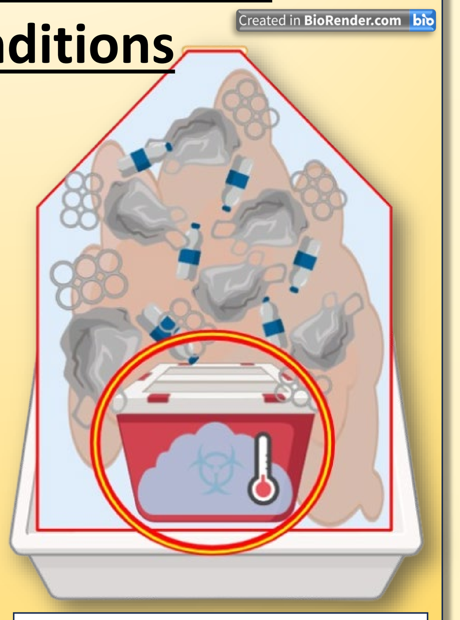


Figure 3. Sharps Container Microclimate Challenge
Sealed containers trap cool air, preventing steam penetration and sterilization.

AUTOCLAVE PROCESS IMPROVEMENTS

- Vacuum cycles for high-challenge waste with minimal water content to remove cool-air pockets.
- Avoid excessive layering and seals (triple or quadruple layers) prior to sterilization.
- To reduce sterilization time, autoclave sharps separately.
- Adding water to bags facilitates steam penetration for liquid and gravity cycles.

Le et al. (2005)

Validation Considerations

- Verify Operational Quality prior to PQ testing.
- Prioritize threshold-volume validations for efficient use of autoclave space and power.
- Use failures to identify steam penetration issues. Adjust only one variable at a time to isolate impediments.
- Suggest evidence-based process improvements:
  - Separation of sharps waste.
  - Excessive bagging or pre-cycle sealing.
  - Alternative cycle parameters.
- Significant autoclave repairs, configuration changes, or failed PQs will reset validation.

Future Directions

Extend PQ validation procedure to all SHL DCD autoclave models with model waste.

Collect evidence to drive process improvements where needed.

Publication of an official validation procedure in the SHL Quality and Operations Records.

Use of data loggers to measure precise conditions in model bags.

References

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