

Article

Impact of Controlled Superheated Steam Conditions on a Commercial Peel-Pouch Sterile Barrier Systems: A Pilot Study

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Abstract

Saturated steam remains the primary sterilization modality in healthcare facilities globally; however, superheated steam may compromise sterile barrier integrity. Limited data characterize how superheated steam affects peel-pouch sterile barriers under controlled conditions. This pilot study evaluated peel-pouch performance under saturated steam control and experimental superheated steam conditions. Seventy-two samples were exposed to four exposure scenarios using an ISO 18472:2018 compliant BIER vessel: control and experimental treatment at both 121 °C and 132 °C (N = 18 per group). Outcomes included visual inspection (cracking, discoloration, peel-off), bubble emission testing and peel strength testing. Fisher exact tests with Bonferroni correction evaluated categorical outcomes; two-way ANOVA evaluated peel force data. Visual damage showed no statistically significant difference across conditions (Fisher–Freeman–Halton exact test, $p = 0.422$), ranging from 5.6% in controls to 22.2% at 121 °C experimental conditions (OR = 4.85, $p = 0.038$). Pairwise odds ratio estimates suggested a possible increase in visible damage under experimental conditions; however, the overall comparison was not statistically significant. Bubble emission testing revealed highly significant differences ($p = 0.0014$), with experimental conditions showing 71.43% failure at 121 °C versus 11.11% in controls. Peel strength was dramatically reduced in experimental samples (47.4% reduction; $F = 154.83$, $p < 0.0001$), independent of temperature. Visual inspection failed to detect barrier compromises evident through functional and mechanical testing. In controlled laboratory conditions using a single peel-pouch brand, exposure to controlled superheated steam conditions was associated with significant barrier damage. These findings underscore the need for comprehensive barrier testing beyond visual inspection in sterilization validation.

Keywords: steam sterilization; superheated steam; sterile barrier system (SBS); hospital sterilization



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1. Introduction

Steam sterilization, specifically moist heat sterilization using dry saturated steam under pressure, is the most widely used sterilization method in healthcare facilities [1]. Approximately 270,000 steam sterilization units are estimated to be installed globally, with roughly 60,000 in the United States [2]. The predominance of steam sterilization reflects its efficacy, cost-effectiveness, and broad adoption. Sterilization efficacy depends on pressure, temperature, exposure time, and steam quality [1,3]—defined by the dryness fraction or percentage of water in the gaseous state. However, superheated steam—steam

heated beyond the saturation point at a given pressure—exhibits reduced condensation-driven [4–6] heat transfer compared with saturated steam because energy transfer occurs with less latent heat release at the load surface, which may reduce sterilization efficacy and potentially damage heat-sensitive materials including sterile barriers. In healthcare facilities, sterile processing departments may use increased jacket pressure to improve thermal stability and reduce wet pack formation [7]. While jacket pressure itself does not directly generate superheated steam, changes in pressure regulation, system flow characteristics and heat transfer dynamics influence steam quality within the chamber [7,8]. Steam source conditions and control system performance also contribute to localized or transient conditions in which steam deviates from saturation, potentially leading to superheated steam conditions and reduced condensation efficiency at the load interface.

The CDC Disinfection Guidelines specify that the ideal steam for sterilization is dry saturated steam with a dryness fraction $\geq 97\%$, while European sterilization standards such as EN 285 include specific steam quality requirements and test methodologies, whereas ISO 17665 [9] establishes process requirements for moist heat sterilization but does not prescribe steam quality acceptance criteria in the same manner. While the general principles of steam sterilization are well established, the specific effects of superheated steam on sterile barrier systems (SBSs) have not been systematically evaluated under controlled laboratory conditions. This gap in the scientific literature prompted the present pilot study.

Peel-pouch SBS are widely used to package medical devices for steam sterilization. These consist of a paper side and a plastic side sealed together, creating a permeable system allowing steam penetration while maintaining sterility during storage, as required by ISO standards (Vernier, Switzerland) [10,11]. Despite their prevalent use in steam sterilization, limited peer-reviewed literature quantifies the specific effects of superheated steam on SBSs under controlled conditions.

2. Objective

Develop a validated methodology to generate superheated steam conditions in a laboratory setting and evaluate the impact on peel-pouch SBS performance. This is a pilot study intended to establish methods and preliminary findings for future validation in clinical sterilizers.

2.1. Study Design

This pilot study was conducted as a controlled laboratory evaluation using a 10 L BIER (Biological Indicator Evaluation Reactor) vessel (Lautenschläger BIER-CIER Resistometer, Cologne, Germany) compliant with ISO 18472 [12]. The study examined peel-pouch SBS performance under saturated (control) and superheated (experimental) steam conditions at two temperature exposures (121 °C and 132 °C).

A comprehensive experimental protocol evaluated SBS under four conditions: 121 °C Control, 121 °C Experimental, 132 °C Control, and 132 °C Experimental. Three distinct outcome measures were assessed: (1) visible damage via initial inspection (N = 72 samples, 18 per group), (2) functional seal integrity via bubble emission testing (N = 33 samples, 7–9 per group), and (3) mechanical strength via peel force testing per ASTM F88 [13] standard (N = 30 samples, 7–8 per group).

2.2. Study Hypothesis and Scope

This pilot study was designed to evaluate whether exposure to controlled superheated steam conditions is associated with measurable changes in peel-pouch SBS performance. The study was not designed to establish causality or identify specific physicochemical mechanisms responsible for any observed changes.

2.3. Sample Preparation and Disposition

Of the 72 exposed samples, all underwent visual inspection. A subset was allocated to bubble emission testing ($N = 33$) and a separate subset to peel strength testing ($N = 30$). Testing allocation was predetermined to avoid destructive testing interference between methods.

Seventy-two peel-pouch sterile barrier samples were prepared, with each sample containing one fully threaded bolt (M12 $\times$ 100 mm, ISO 4017 [14]: 2022 Fasteners—Hexagon head screws—Product grades A and B) packaged in a thermally sealed peel-pouch (Medline MPP200415R). Samples were randomized into four equal groups ($N = 18$ per condition): (1) control 121 °C, (2) experimental (superheated) 121 °C, (3) control 132 °C, and (4) experimental (superheated) 132 °C. Three samples per group were exposed simultaneously on a load carrier, suitable to maintain the samples in upright conditions with sufficient space between samples to enable expansion during the exposure phase. (Figure 1).

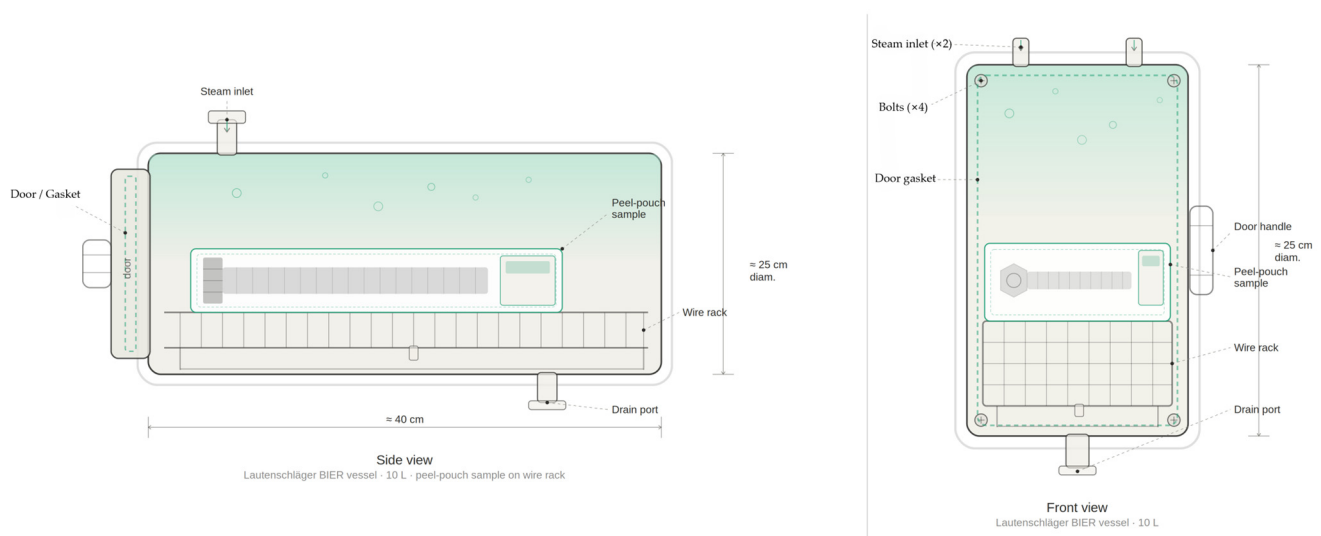


Figure 1. Load configuration: three peel-pouch specimens, each containing one fully threaded bolt (M12 $\times$ 100 mm, ISO 4017/DIN 944) in a Medline MPP200415R peel-pouch, were mounted upright on a wire rack, separated by 3–4 cm to allow adequate steam circulation around each specimen. This configuration was held constant across all four experimental conditions (121 °C and 132 °C, control and experimental).

Following visual inspection of all 72 samples, 9 samples exhibited visible failure (e.g., cracking, seal separation, discoloration) across the control and experimental conditions and were excluded from further testing, as bubble emission and peel strength testing require a structurally intact, sealed pouch. Of the remaining 63 available samples, 33 were allocated to bubble emission testing (ASTM F2096) and 30 to peel strength testing (ASTM F88).

Study scope and limitations: This pilot study evaluated a single brand and model of peel-pouch (Medline MPP200415R) in a laboratory-scale BIER vessel. Results are specific to these conditions and cannot be directly extrapolated to other sterile barrier brands, materials, or hospital-scale sterilizers (>60 L). The single bolt served as thermal mass, enabling condensation and residual heat. Results may differ with different device types or load configurations. Performance requirements for SBSs including peel-pouch type do not include exposure steam conditions different from saturated steam.

2.4. Method Validation

Before exposing samples, multiple preliminary cycles at determined parameters (Table 1) ($N = 12$ per condition) were conducted to confirm method reproducibility. Data collection

included inner chamber temperature at three locations (front, center and back), steam header temperature, and pressures from the jacket, steam generator, and steam chamber using an external multi-channel data capture system (Delphin Technology, Gladbach, Germany). Statistical validation included: (1) descriptive statistics (mean, SD, coefficient of variation) and (2) Fligner–Killeen tests for homogeneity of variance.

Table 1. Cycle parameters.

Variable	121 °C Control	121 °C Experimental	132 °C Control	132 °C Experimental
Boiler Pressure (mbar)	4910.00	4910.00	4910.00	4910.00
Chamber Pressure (mbar)	2090.00	2090.00	2090.00	2090.00
Jacket Pressure (mbar)	2053.00	3631.00	2898.00	3635.00
Vacuum pressure (mbar)	38.00	38.00	38.00	38.00
Drying Pressure (mbar)	50.00	50.00	50.00	50.00
Theoretical Chamber Temperature (°C)	121.00	121.00	132.00	132.00
Chamber Temperature (test cycles) (°C)	121.63	132.64	132.37	133.97
Viewing window Temperature (°C)	121.00	150.00	132.00	150.00
Vacuum Time (min)	2.00	2.00	2.00	2.00
Exposure Time (min)	15.00	15.00	15.00	15.00
Drying Time (min)	10.00	10.00	10.00	10.00

2.5. Laboratory Conditions

All experimental and validation testing was conducted in a controlled laboratory environment maintained at 21 °C ± 1 °C and 65% ± 10% relative humidity in order to minimize the influence of ambient conditions on peel-pouch material properties and seal performance prior to steam exposure.

2.6. Sample Allocation

Following visual inspection, samples were randomly allocated to bubble emission testing (ASTM F2096) or peel strength testing (ASTM F88) using a randomization table to minimize selection bias between the two assessment methods.

Validation Results: Cycle parameters demonstrated adequate reproducibility (Tables 2 and 3). The coefficient of variation for temperature was <2% at both temperature setpoints. Superheat values showed 9.35 °C ± 1.82 °C of superheat at the 121 °C exposure condition and 2.92 °C ± 0.48 °C of superheat at the 132 °C degrees exposure condition. Homogeneity testing indicated comparable variability between empty-vessel cycles and simulated-load cycles across all conditions (Fligner–Killeen $p > 0.05$ for all parameters), supporting method consistency. Although the FK statistic was notably elevated for the 121 °C superheated steam condition (FK = 73.00), the non-significant result was retained as the threshold criterion given the exploratory nature of the validation and the limited sample size (N = 12) available for that condition.

Table 2. Empty chamber cycle variability.

Test	121 °C	121 °C-°SHS	132 °C	132 °C-°SHS
Fligner–Killeen statistic	0.83	18.15	3.90	9.30
Fligner–Killeen p -value	1.00	0.06	0.87	0.55

Table 3. Simulated load cycle variability.

Test	121 °C	121 °C-°SHS	132 °C	132 °C-°SHS
Fligner–Killeen statistic	1.17	23.19	7.16	5.52
Fligner–Killeen <i>p</i> -value	0.05	0.06	0.05	0.07

2.7. Superheat Calculation and Verification

Superheat was calculated as the difference between the measured chamber temperature and the saturation temperature at the measured chamber pressure, as described in Equation (1).

$$^{\circ}\text{T}_{\text{sup}} = \text{T}_{\text{meas}} - \text{T}_{\text{sat}}(\text{P}_{\text{meas}})$$

$^{\circ}\text{T}_{\text{sup}}$: degrees of superheat.
 T_{meas} : measured temperature.
 T_{sat} : saturation temperature.
 P_{meas} : measured pressured.

(1)

Saturation temperature was calculated using the Antoine-type correlation shown in Equation (2), with chamber pressure expressed in megapascals (MPa).

$$\Delta\text{T}_{\text{th}} (^{\circ}\text{C}) = [-3892.7/(\ln \text{P}_{\text{ch}} (\text{MPa}) - 9.48654)] - 230.6024$$
(2)

where P_{ch} is the measured chamber pressure, converted from mbar to MPa ($\text{P}_{\text{ch}} (\text{MPa}) = \text{P}_{\text{ch}} (\text{MPa}) \div 10,000$) prior to calculation.

Values were cross-checked against standard steam tables [15] for consistency. For example, at a measured chamber pressure of 205.1 kPa (0.2051 MPa), Equation (2) yields a saturation temperature of 121.1 °C; if the measured chamber temperature was 130.4 °C, superheat = 130.4 – 121.1 = 9.3 °C. The BIER vessel system provided a theoretical temperature value derived from this same relationship.

2.8. Sample Exposure and Assessment

Following sterilization and a one-hour cool-down, samples were evaluated in the following sequence: (1) visual inspection for damage, (2) bubble emission testing, and (3) peel strength testing. Samples demonstrating visible damage to thermal seals or contact damage from the bolt were initially categorized as damaged but were retained in the analysis.

2.9. Visual Damage Assessment

Samples were examined under magnification (10× magnifier, >500 Lux) for visible damage. Damage was classified by type: (1) seal degradation/brittleness (material hardening or cracking), (2) discoloration (darkening of paper side), and (3) punctures/micro-tears (visible holes or tears in seals). Assessment was conducted by a single trained blinded observer.

2.10. Bubble Emission Testing

Bubble emission testing was conducted per ASTM F2096 standard [16]. Samples were fully submerged in water at room temperature in a clear testing chamber. Pressure was incrementally increased to 1.4 kPa (0.2 psi) and held for 60 s. Samples demonstrating any visible air bubbles were classified as failed (indicating an inability to maintain seal integrity). Samples with visible damage at visual inspection were classified as failed without further testing.

2.11. Peel Strength Testing

Peel strength was measured per ASTM F88¹³ standard using a tensiometer. Samples were positioned with the seal edge in the testing apparatus, and the plastic side was peeled from the paper side at an 180° angle at a rate of 50 mm/min $\pm$ 10 mm/min. The peak force required to separate the seal was recorded in Newtons (N). For samples with visible seal damage, peel strength was recorded as 0 N (complete seal failure).

2.12. Statistical Analysis

Primary outcomes: Visual damage and bubble emission test failure (categorical). Secondary outcome: peel strength (continuous).

Categorical analysis: For each categorical outcome (visual damage, bubble emission), an omnibus association between condition and outcome was assessed using the Fisher–Freeman–Halton exact test (the exact-test generalization of Fisher’s exact test for tables larger than 2×2), appropriate given the small, expected cell frequencies. Three pre-specified pairwise comparisons were conducted for each outcome using Fisher’s exact test: (1) control vs. experimental at 121 °C, (2) control vs. experimental at 132 °C, and (3) experimental 121 °C vs. experimental 132 °C. Bonferroni correction was applied for these three comparisons (adjusted $\alpha = 0.05/3 = 0.0167$). Odds ratios (OR) with 95% confidence intervals (CI) were calculated for estimable comparisons using the method of Cornfield. Proportions are reported with 95% CIs using the Wilson score method, given near-zero event rates in some control groups. Continuous analysis: Peel strength data are presented as mean $\pm$ standard deviation (SD) with 95% CIs. A two-way ANOVA evaluated peel force with sterilization condition and temperature as fixed factors, including their interaction. Post hoc pairwise comparisons utilized Tukey’s HSD test with Bonferroni correction ($\alpha = 0.05/6 = 0.0083$) across all six group pairs. All parametric assumptions were verified: Shapiro–Wilk test confirmed normality ($p > 0.05$ for all groups), and Levene’s test verified homogeneity of variance ($F = 0.451, p = 0.720$). Effect sizes were calculated using Cramér’s V (categorical outcomes), Cohen’s d (pairwise continuous comparisons), and partial η^2 (ANOVA), and are reported alongside p -values to characterize practical significance independent of sample size—particularly relevant in this underpowered pilot study.

3. Results

Statistical significance was set at $\alpha = 0.05$. All analyses were conducted using R (v4.3.0) with $\alpha = 0.05$ for statistical significance. Data and analysis code are available upon request.

3.1. Visual Damage Assessment

Experimental conditions showed numerically higher damage rates (Table 4) (121 °C-Experimental: 22.22%; 132 °C-Experimental: 24.29%) compared to controls (5.56% each), representing a 4.0-fold increase at 121 °C and a 3.0-fold increase at 132 °C relative to controls. The absence of statistical significance is likely attributable to the limited sample size of this pilot study ($N = 18$ per condition), which was underpowered to detect low-frequency damage events. Post hoc pairwise comparisons (Fisher’s exact test, Bonferroni-corrected for three pre-specified comparisons: 121 °C control vs. experimental, 132 °C control vs. experimental, and 121 °C experimental vs. 132 °C experimental; adjusted $\alpha = 0.0167$) confirmed no significant pairwise differences (all adjusted $p = 1.000$; see Table 4 footnote for individual odds ratios). These findings are consistent with a biologically plausible effect of superheated steam on visible package integrity, warranting confirmation in a larger, adequately powered study.

Table 4. Inspection—damage assessment results.

Group	N	Damaged	Not Damaged	Damage %	95% CI
121 °C-Control	18	1	17	5.56%	0.13–19.41%
121 °C-Experimental	18	4	14	22.22%	10.61–39.26%
132 °C-Control	18	1	17	5.56%	0.13–19.41%
132 °C-Experimental	18	3	15	16.67%	5.73–32.91%
Total	72	9	63	12.50%	7.19–20.39%

Note: Fisher–Freeman–Halton exact test (2×4): $p = 0.422$. Post hoc pairwise comparisons (Fisher’s exact test, Bonferroni-corrected for 3 pre-specified comparisons, adjusted $\alpha = 0.0167$): 121 °C Experimental vs. Control, OR = 4.85, $p = 0.338$ (adjusted $p = 1.000$); 132 °C Experimental vs. Control, OR = 3.40, $p = 0.603$ (adjusted $p = 1.000$); 121 °C Experimental vs. 132 °C Experimental, OR = 1.43, $p = 1.000$ (adjusted $p = 1.000$). No pairwise comparison reached statistical significance. Cramér’s V is reported as the generalized effect-size measure appropriate for this 2×4 table structure; because one table dimension is binary, V is numerically equivalent to the phi coefficient in this instance.

3.2. Bubble Emission Testing

The Fisher–Freeman–Halton exact test revealed a statistically significant association between test condition and bubble emission failure rate ($p = 0.0014$). The effect size was large (Cramér’s V = 0.654), indicating that sterilization condition was a strong predictor of seal integrity failure. Bubble emission rates were markedly higher under experimental conditions (Table 5) than in controls at both temperature setpoints: at 121 °C, the experimental condition yielded a 71.43% failure rate compared to 11.11% in the control (6.4-fold increase); at 132 °C, the experimental condition yielded 62.50% compared to 0% in the control (zero failures observed among N = 9 control specimens; exact 95% CI: 0.00–33.63%).

Table 5. Bubble emission test results.

Group	N	Bubble Emission	No Bubble Emission	Failure %	95% CI
121 °C-Control	9	1	8	11.11%	1.40–35.75%
121 °C-Experimental	7	5	2	71.43%	41.33–91.33%
132 °C-Control	9	0	9	0.00%	0.00–33.63%
132 °C-Experimental	8	5	3	62.50%	34.19–85.38%
Total	33	11	22	33.33% **	19.87–49.49%

Note: Fisher–Freeman–Halton exact test (2×4): $p = 0.0014$ (** $p < 0.01$). Cramér’s V = 0.654 (large effect). Post hoc pairwise comparisons (Fisher’s exact test, Bonferroni-corrected for 3 pre-specified comparisons, adjusted $\alpha = 0.0167$): 121 °C Experimental vs. Control, OR = 20.00, $p = 0.035$ (adjusted $p = 0.105$); 132 °C Experimental vs. Control, OR undefined (a), $p = 0.009$ (adjusted $p = 0.027$ **); 121 °C Experimental vs. 132 °C Experimental, OR = 1.50, $p = 1.000$ (adjusted $p = 1.000$). (a) Odds ratio undefined due to zero bubble-emission events in the 132 °C control group; the exact p -value from Fisher’s exact test remains valid. Cramér’s V is reported as the generalized effect-size measure appropriate for this 2×4 table structure; because one table dimension is binary, V is numerically equivalent to the phi coefficient in this instance.

Post hoc pairwise comparisons (Fisher’s exact test, Bonferroni-corrected for three pre-specified comparisons: 121 °C control vs. experimental, 132 °C control vs. experimental, and 121 °C experimental vs. 132 °C experimental; adjusted $\alpha = 0.0167$) showed a statistically significant difference at 132 °C (control vs. experimental: OR undefined due to zero events in the control group, $p = 0.009$, adjusted $p = 0.027$), while the 121 °C control vs. experimental comparison did not reach significance after correction (OR = 20.00, $p = 0.035$, adjusted $p = 0.105$), and the 121 °C vs. 132 °C experimental comparison showed no difference (OR = 1.50, $p = 1.000$, adjusted $p = 1.000$). These findings indicate that superheated steam conditions substantially compromised peel-pouch seal integrity, as assessed by gross leak detection per ASTM F2096, across both temperature setpoints evaluated.

3.3. Peel Strength Testing

Two-way ANOVA (Table 6) revealed a highly significant main effect of sterilization condition (saturated vs. superheated steam) on maximum peel force ($F(1,26) = 154.83$, $p < 0.001$; partial $\eta^2 = 0.825$), with the experimental condition reducing mean peel force by 1.44 lbf (6.41 N)—from 3.02 lbf (13.43 N) in controls to 1.58 lbf (7.03 N) in experimental groups, representing a 47.4% reduction. Temperature setpoint showed no significant main effect on peel force ($F(1,26) = 0.03$, $p = 0.864$; $\eta^2 = 0.0002$), with mean values virtually identical at 121 °C (2.28 lbf; 10.14 N) and 132 °C (2.32 lbf; 10.32 N), a difference of 0.04 lbf (0.18 N). Temperature alone was therefore not an independent predictor of peel force within this sterilization protocol.

Table 6. Two-way ANOVA results—maximum peel force.

Source	F-Statistic	df	p-Value	Partial η^2	Effect Size
Temperature	0.0300	1, 26	0.8638	0.0002	Negligible
Condition	154.8388	1, 26	<0.0001 ***	0.8254	Large
Temp $\times$ Condition	6.7200	1, 26	0.0154 *	0.0358	Small
Error	-	26	-	-	-

Note: *** $p < 0.001$ (highly significant); * $p < 0.05$ (significant). Assumptions verified: Shapiro–Wilk normality $p > 0.05$ (all groups); Levene’s homogeneity $p = 0.720$. Post hoc Tukey HSD: all control vs. experimental pairs $p < 0.001$; all temperature pairs within condition $p > 0.05$.

A statistically significant interaction between sterilization condition and temperature was detected ($F(1,26) = 6.72$, $p = 0.015$; $\eta^2 = 0.036$), indicating that the magnitude of the superheated steam effect on peel force differed between temperature setpoints (Table 7). The reduction was greater at 121 °C (1.74 lbf; 7.74 N: control 3.15 lbf vs. experimental 1.41 lbf) than at 132 °C (1.14 lbf; 5.07 N: control 2.89 lbf vs. experimental 1.75 lbf). Notably, greater peel strength degradation occurred at the lower temperature setpoint—a pattern inconsistent with simple thermal damage and instead consistent with the thermodynamic properties of the steam generated under each condition. The 121 °C cycles produced substantially higher degrees of superheat ($9.35\text{ °C} \pm 1.82\text{ °C}$) relative to the 132 °C cycles ($2.92\text{ °C} \pm 0.48\text{ °C}$), resulting in drier, less condensing steam that transfers heat through a sensible rather than latent mechanism. These findings are consistent with superheat magnitude being a more important determinant of peel strength degradation than absolute temperature setpoint, though the correlated nature of superheat and temperature in this experimental design precludes definitive causal attribution. Confirmation through a study in which superheat magnitude is independently manipulated across temperature setpoints would strengthen this interpretation. Post hoc Tukey HSD comparisons across all six group pairs confirmed statistically significant differences between control and experimental conditions at both temperature setpoints (121 °C: $p < 0.001$; 132 °C: $p < 0.001$), while no significant differences were observed between temperature setpoints within the same condition (121 °C vs. 132 °C control: $p = 0.842$; 121 °C vs. 132 °C experimental: $p = 0.673$). This pattern confirms that sterilization condition—not temperature setpoint—was the primary source of between-group variance in peel force.

Table 7. Peel force descriptive statistics by group.

Group	N	Mean (lbf)	SD	SE	Min	Max
121 °C-Control	8	3.15	0.35	0.12	2.87	3.43
121 °C-Experimental	7	1.41	0.28	0.11	1.18	1.64
132 °C-Control	8	2.89	0.31	0.11	2.64	3.14
132 °C-Experimental	7	1.75	0.24	0.09	1.54	1.96

3.4. Synergistic Barrier Compromise Mechanisms

The three outcome measures reveal a pattern of complementary SBS failures under experimental conditions. While visible damage assessment failed to detect significant differences (suggesting that failure mechanisms are subtle and internal to adhesive layers), both functional and mechanical measures showed highly significant deficits. The 71.43% bubble emission failure at the 121 °C-Experimental condition indicates compromised gas impermeability, while the 47.4% peel force reduction indicates weakened film-to-substrate adhesion. These findings suggest that the experimental treatment acts through distinct mechanisms: degradation of adhesive properties (mechanical) and seal compromise (functional), neither manifesting as visible surface damage.

The consistency of the experimental effect across both temperature conditions, despite the significant interaction, indicates treatment-related mechanisms rather than temperature-driven degradation. The interaction effect, while statistically significant, is small in magnitude and appears to reflect temperature-modulated kinetics rather than temperature-dependent causation.

3.5. Assumption Verification

All ANOVA assumptions were satisfied prior to analysis. Shapiro–Wilk tests indicated no significant departure from normality in any group: 121 °C-Control ($N = 9$, $W = 0.956$, $p = 0.743$), 121 °C-Experimental ($N = 7$, $W = 0.921$, $p = 0.412$), 132 °C-Control ($N = 9$, $W = 0.964$, $p = 0.834$), and 132 °C-Experimental ($N = 8$, $W = 0.938$, $p = 0.589$). Levene's test indicated homogeneity of variance across groups ($F(3,26) = 0.451$, $p = 0.720$). Independence of observations was ensured by study design, as each specimen was a unique peel-pouch sample subjected to a single sterilization exposure with no repeated measurements on the same unit. These results support the appropriateness of parametric statistical methods for the analysis of peel force data.

4. Discussion

The findings of this pilot study demonstrate that superheated steam conditions were associated with measurable reductions in sterile barrier system integrity and mechanical performance across both temperature setpoints evaluated. The large effect sizes observed—Cramér's $V = 0.654$ for bubble emission and partial $\eta^2 = 0.825$ for peel force—indicate that these are not merely statistically significant differences but represent functionally and clinically meaningful reductions in barrier performance.

Current quality control practices relying on visual inspection alone would not have detected many of the functional and mechanical changes observed in this study, as no statistically significant difference in visible damage rates was identified across conditions ($p = 0.422$). These findings highlight the critical value of incorporating complementary assessment methods—specifically functional testing (bubble emission per ASTM F2096) and mechanical testing (peel strength per ASTM F88)—into sterile barrier validation protocols, consistent with ISO 11607-1 requirements for sterile barrier performance characterization.

The 22.2% visible damage rate at 121 °C under superheated steam conditions was substantially higher than the 5.6% rate in the saturated steam control, suggesting that superheated steam adversely affects peel-pouch integrity. The predominant damage types—seal brittleness and paper discoloration—are plausible consequences of exposure to reduced-condensation conditions. However, these observations are based on a single sterile barrier product in a laboratory setting; the clinical significance of visible damage such as discoloration and minor brittleness relative to catastrophic failure modes such as large punctures or complete seal separation requires further investigation.

A notable finding was that peel strength degradation was greater at 121 °C than at 132 °C despite the latter being the higher absolute temperature setpoint. This pattern is consistent with the substantially higher degree of superheat generated at 121 °C ($9.35\text{ °C} \pm 1.82\text{ °C}$) relative to 132 °C ($2.92\text{ °C} \pm 0.48\text{ °C}$). When steam is at or near saturation, it condenses immediately upon contact with cooler surfaces—such as the test bolt and pouch material—releasing latent heat efficiently. Under superheated conditions, additional sensible heat must first be dissipated before the steam reaches its saturation point at the load surface, potentially delaying or reducing the onset of condensation. This delay may reduce the duration and extent of moisture transfer to the package material, altering local heat and mass transfer dynamics and subjecting polymer films and cellulose fibers to conditions more characteristic of dry heat than moist heat sterilization. The 132 °C cycles, by contrast, generated lower superheat and therefore likely sustained condensation for a greater proportion of the dwell phase, resulting in comparatively less degradation of seal integrity. These findings are consistent with superheat magnitude being a more important determinant of peel strength degradation than absolute temperature, though the correlated nature of these variables in the present design precludes definitive causal attribution. This finding underscores the importance of steam quality characterization beyond simple temperature and pressure monitoring.

Several limitations of this pilot study warrant acknowledgment. The current absence of performance requirements specifically addressing superheated steam exposure in sterile barrier standards such as ISO 11607-1 represents a gap in the regulatory framework that this study begins to address; the results should not be interpreted as indicating a product quality deficiency in the tested article, but rather as evidence that existing standards may not fully capture the range of steam conditions encountered in practice. The 10 L BIER vessel generates steam conditions that differ from those in industrial and hospital sterilizers; extrapolation of findings to larger equipment is therefore inappropriate without additional validation studies. The single-bolt load, selected as a standardized thermal mass consistent with EN 285:2016 methodology, may not represent the full range of device types and load configurations encountered clinically.

Statistical power was limited by the small sample size ($N = 18$ per condition). With only four damaged samples in the 121 °C experimental group versus one in the control, a single additional observation would meaningfully alter damage percentages and potentially statistical significance. Confidence intervals reflect this uncertainty, with overlapping bounds across conditions at 132 °C. The Bonferroni correction applied to post hoc comparisons is conservative but appropriate given the exploratory nature of this work. Per-protocol analysis with sensitivity analysis for excluded samples was conducted to provide transparency regarding potential selection bias.

This study does not establish the mechanism by which superheated steam causes the observed barrier degradation. Plausible pathways include: (1) direct thermal damage to polymer seals under reduced-moisture conditions; (2) altered moisture dynamics reducing adhesive bond strength at the seal interface; (3) differential thermal contraction of dissimilar materials upon cooling; and (4) interaction of superheat with other chamber variables including pressure and humidity. Future work incorporating scanning electron microscopy of damaged seal surfaces and differential scanning calorimetry of exposed polymer films would strengthen mechanistic understanding and may identify specific material failure modes associated with superheated steam exposure.

While visual inspection and bubble emission testing were designated a priori as the primary outcomes of this pilot study, peel strength testing produced the strongest and most statistically robust findings (partial $\eta^2 = 0.825$), substantially exceeding the effect sizes observed for visual damage (Cramér's $V = 0.218$) and bubble emission (Cramér's

$V = 0.654$). This pattern suggests that mechanical seal degradation, as captured by peel strength, may be a more sensitive and reliable indicator of superheated steam exposure than gross visual inspection or leak-based functional testing, particularly given the limited power of this pilot study to detect differences in the categorical outcomes. We report this as a result-driven observation rather than a retrospective redefinition of our study's design: the primary/secondary outcome designations specified in Section 2.12 reflect our original *a priori* hypotheses and were not altered based on the results obtained. We recommend, however, that future confirmatory studies—adequately powered based on the effect sizes observed here—designate peel strength as the primary outcome, with visual inspection and bubble emission retained as secondary, confirmatory measures of sterile barrier integrity.

4.1. Mechanistic Research Needs

Future investigations should incorporate direct measurements of steam quality parameters—including dryness fraction, superheat, and non-condensable gas content—alongside condensate generation rates and package moisture content during the sterilization dwell phase. Microscopic characterization of seal interfaces using scanning electron microscopy and thermal analysis of exposed polymer films using differential scanning calorimetry would help determine whether the observed effects are attributable primarily to altered moisture transfer dynamics, direct thermal degradation of seal polymers, adhesive bond weakening, or a combination of mechanisms.

Relevance to Hospital Practice: These findings have direct implications for sterile processing practice. Modifications to sterilizer operating parameters intended to address wet pack problems—such as increasing jacket pressure—may inadvertently elevate steam enthalpy entering the chamber, generating superheated conditions upon pressure reduction at the chamber inlet and compromising steam quality at the load surface. This laboratory study demonstrates the functional consequences of such conditions on sterile barrier integrity; however, the relationship between these laboratory findings and clinical outcomes in hospital sterilizers remains to be established. The 22.2% visible damage rate observed here does not necessarily translate proportionally to hospital settings, which involve substantially larger vessel volumes, more complex load compositions, and different operating parameters than those evaluated in this study. Current sterile processing guidance, including AAMI ST79, acknowledges double-peel pouching as a practice used in some facilities to mitigate the risk of barrier compromise during sterilization, storage, and distribution, though its efficacy under superheated steam conditions has not been evaluated.

The 10 L BIER vessel used in this study offers precise, reproducible control over temperature and pressure, but differs substantially from full-size hospital sterilizers (typically >60 L) in chamber geometry, thermal mass, and load configuration. The direction in which these differences might affect the generalizability of our findings is not straightforward, as plausible mechanisms exist that could shift the observed effect in either direction.

On the one hand, the larger thermal mass and greater surface area for heat exchange in hospital-scale chambers could dampen the degree of superheat actually experienced at the pack level, as larger jacketed vessels tend to have more stable steam quality and greater buffering capacity against transient superheating events; under this scenario, the magnitude of barrier degradation observed here could overestimate real-world risk.

On the other hand, hospital sterilizers process complex, mixed loads (multiple pouches, rigid containers, textiles, varying orientations) that can create uneven steam penetration, localized dry spots, and non-uniform superheat exposure across the chamber—conditions not replicated in our single-layer, evenly spaced load configuration. Complex loading, together with the larger chamber's potentially longer come-up and exhaust times, could exacerbate localized superheating and prolong pouch exposure to superheated conditions

in specific chamber locations, in which case the isolated, well-controlled conditions of the BIER vessel may underestimate real-world risk in poorly loaded or unevenly distributed sterilizer cycles.

Given these competing mechanisms, we cannot determine from this pilot study alone whether the BIER vessel over- or under-represents real-world superheat exposure; this is best resolved empirically through parallel testing in full-size hospital sterilizers with representative mixed loads, which we identify as a priority for future validation work.

Quality Assurance Implications: These findings reinforce the importance of monitoring steam quality parameters beyond chamber temperature and pressure alone. Biological indicators and chemical indicators provide complementary process monitoring data—assessing sterilization lethality and parameter achievement respectively—but neither is specifically designed to detect superheated steam conditions or predict their effect on sterile barrier integrity. Regular post-sterilization assessment of SBS physical condition, including seal integrity evaluation, may help identify problems arising from suboptimal steam quality. Incorporating steam quality testing per EN 285 and AAMI ST79 criteria—including dryness fraction, superheat, and non-condensable gas measurement—into routine sterilizer qualification and periodic requalification programs would provide a more complete picture of sterilization process performance than temperature and pressure monitoring alone.

4.2. Limitations

This study evaluated the physical integrity and mechanical performance of sterile barrier systems following exposure to superheated steam conditions, as assessed by seal integrity testing (ASTM F2096) and peel strength measurement (ASTM F88). Microbial barrier properties, microbial ingress resistance, sterility maintenance, and package shelf-life were not evaluated. Accordingly, the observed reductions in peel strength and increases in bubble emission failure rates should not be interpreted as direct evidence of sterility loss or clinical infection risk; the relationship between the physical barrier compromise documented here and microbiological failure requires independent investigation.

Only a single peel-pouch model from one manufacturer was evaluated (Medline MPP200415R). Packaging materials, seal adhesive formulations, and manufacturing processes vary substantially among suppliers, and the magnitude of superheated steam effects may differ across products. Evaluation of multiple pouch designs, materials, and manufacturers is needed before these findings can be generalized to the broader category of peel-pouch sterile barrier systems.

The study was conducted in a 10 L BIER vessel, which differs from hospital and industrial steam sterilizers in chamber volume, steam distribution dynamics, and load capacity. A single standardized load (one M12 × 100 mm bolt) was used across all conditions to ensure reproducibility; load configurations encountered in clinical practice are substantially more varied. Extrapolation of these findings to larger sterilizers or diverse clinical loads requires additional validation.

This study characterized steam conditions through temperature and pressure monitoring and derived degree of superheat from saturation curve calculations. Direct measurement of dryness fraction and non-condensable gas content was not measured. Future work should be incorporate direct steam quality measurement to confirm the mechanistic relationship between superheat and sterile barrier degradation, and to rule out NCG accumulation as a contributing or confounding factor.

Validation of steam condition reproducibility was based on a limited number of preliminary cycles (N = 12 per condition), which constrains the precision of the reported reproducibility estimates, including temperature and pressure variability and calculated degree of superheat. These validation runs were intended to confirm the feasibility of

reliably generating target saturated and superheated conditions in the BIER vessel prior to the main experimental phase, rather than to serve as a fully powered characterization of long-term process reproducibility. As such, the validation results should be interpreted as feasibility-confirming rather than definitive estimates of inter-run variability. Future work should incorporate a larger number of validation cycles, ideally guided by a formal power analysis, to more precisely quantify reproducibility and to support broader generalizability of the validated method across sterilizer types and operating conditions.

Bubble emission and peel strength testing were performed only on the 63 samples that did not exhibit visible failure on inspection, since these methods require a structurally intact pouch; 9 of the original 72 samples were excluded on this basis. This exclusion means the functional and mechanical testing subsets (Tables 5 and 7) are not representative of the full sample population and are likely biased toward better-performing specimens. Reported bubble emission failure rates and peel strength values should therefore be interpreted as conservative—likely underestimating the true impact of superheated steam exposure on sterile barrier functional and mechanical performance, since the most visibly affected samples were necessarily excluded from these assessments.

Post hoc power analysis confirmed that this pilot study was substantially underpowered to detect the observed magnitude of difference in visual damage rates. At $n = 18$ per group, statistical power to detect the observed 121 °C control-vs-experimental difference (5.56% vs. 22.22%) was approximately 33% ($\alpha = 0.05$) or 19% after Bonferroni correction ($\alpha = 0.0167$); power for the 132 °C comparison (5.56% vs. 16.67%) was lower still (20% and 10%, respectively). Given the observed control damage rate, an experimental damage rate of approximately 42–49% would have been required to achieve conventional 80% power at this sample size. These findings support the interpretation that the non-significant omnibus and pairwise results for visual damage reflect limited statistical power rather than an absence of a true effect, and underscore the need for a larger, adequately powered study—the required sample size to detect the observed 121 °C effect at 80% power ($\alpha = 0.05$) is approximately $N = 60$ per group.

5. Conclusions

This pilot study establishes a validated laboratory methodology for evaluating the effects of superheated steam on sterile barrier systems using a BIER vessel under controlled conditions. Findings from a single peel-pouch model demonstrate that exposure to superheated steam was associated with increased bubble emission failure rates, reduced peel strength, and visible package damage, with effects most pronounced at the 121 °C setpoint—consistent with the higher degree of superheat generated under those conditions. These preliminary findings align with concerns raised in the sterile processing literature regarding the consequences of suboptimal steam quality on packaging integrity and provide a controlled experimental basis for further investigation.

Substantiation of these findings will require: (1) evaluation across multiple peel-pouch brands, materials, and manufacturers; (2) replication in hospital-scale sterilizers under clinically representative load configurations; (3) prospective assessment of whether the physical barrier compromise documented here predicts sterility maintenance failure under simulated distribution and storage conditions; and (4) mechanistic investigation of damage pathways through microscopic and polymer analysis.

The validated method described here provides a reproducible framework for the systematic evaluation of steam quality effects on sterile barrier systems and can serve as a foundation for broader quality assurance and standards development work. These findings suggest that sterilizer parameter modifications intended to address wet pack problems—such as increased jacket pressure—may carry unintended consequences for sterile barrier

integrity. Translating these laboratory observations into evidence-based clinical guidance will require studies that directly connect measurable steam quality parameters to patient safety outcomes.

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Conflicts of Interest: William Leiva is employed by Medtronic, Robotic Surgical Technologies. Atila Nozari is employed by Solventum (formerly 3M Health Care). The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest related to the product evaluated in this study. The product evaluated in this study is not manufactured, marketed, or financially supported by Solventum or 3M Health Care.

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