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Risk-Based Autoclave Steam Sterilization Cycle Design and Validation for Mixed Biopharmaceutical Loads: Thermodynamic Mapping Integrated with FMEA

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Abstract *— The article addresses the development and validation of risk-based steam sterilization cycles for mixed biopharmaceutical loads by integrating thermodynamic mapping with FMEA methodology. The relevance of the work is driven by increasingly stringent regulatory expectations and the limitations of template autoclave cycles, which do not ensure reliable air removal or the uniform attainment of sterilizing conditions in complex, porous, and combined loads. The aim of the study is to establish a scientifically justified model for cycle design that ensures not only the attainment of the required lethality and SAL levels, but also their robust defensibility before regulators. Methodologically, the work is grounded in the Quality by Design paradigm and a mixed-methods research design that includes high-frequency thermal mapping using wireless data loggers in typical mixed loads, lethality calculation, and a modified FMEA that directly links cycle parameters (vacuum pulses, heating rate, equilibration time) to specific failure modes. It is shown that an unsuccessful cycle is characterized by thermodynamic instability, persistent air pockets, and failure to reach the minimum temperature of 121°C at the critical point, whereas an optimized cycle with intensified vacuuming exhibits rapid equilibration (<15 s), a narrow temperature corridor of 121–124°C, and excess lethality (F0≈29–32 min) that substantially exceeds the overkill criterion. The scientific novelty lies in treating the mixed load as a dynamic risk object and in proposing a hierarchy of acceptance criteria, placing primary emphasis on physical indicators (P/T correlation, equilibration time, absence of cold spots), with integral lethality used as a derived parameter. The article is intended for validation engineers, quality assurance specialists, and biopharmaceutical process developers involved in the design and defense of steam sterilization cycles.*

Keywords *— **steam sterilization, autoclaving, quality risk management, ISO 17665-1:2024, sterility assurance, lethality***

I. INTRODUCTION

Sterilization with saturated steam (autoclaving) has historically been the dominant method of terminal sterilization and component sterilization in the pharmaceutical industry due to its high effectiveness, economic efficiency, and absence of toxic residues (Agalloco, 2024). However, the apparent simplicity of the process, exposure to 121°C for a specified time, conceals complex thermodynamic interactions, particularly when processing heterogeneous, porous, or structurally complex loads (Feurhuber et al., 2023).

Apart from the meaningful change in regulation in the last 10 years, with the publication of Annex 1 to the EU GMP guideline and the revision of ISO 17665-1:2024, the focus is now on process understanding and Quality Risk Management (QRM), both of which can

support continuous Quality improvement (PMS, 2022; ISO, 2024). This range indicates a tight contemporary regulatory authorities, such as the FDA and EMA, which are no longer satisfied with merely demonstrating that a cycle has passed; they require scientific justification of why it passed and how its reliability is ensured under process variability (Marschall et al., 2022).

The relevance of this study stems from the critical need to revise approaches to cycle development. The traditional practice of using template parameters (e.g., three pre-vacuum pulses and 15 minutes holding time) often leads to hidden failures such as the formation of air pockets in instrument cavities or migration of cold spots, creating risks for patient safety, particularly in the manufacture of highly potent products (Chakraborty et al., 2020).

The key problem lies in the disconnection between the physics of heat and mass transfer and the formalism of validation procedures. Existing methods often treat the autoclave load as a static thermal mass, ignoring the dynamic behavior of the steam–air mixture (Feurhuber et al., 2023).

Standard protocols frequently assume that temperature and pressure are rigidly correlated via the saturation curve; however, the presence of non-condensable gases (NCGs) disrupts this relationship (Dalton's law), resulting in ineffective sterilization even when the target temperature has been reached (Miguel et al., 2022).

Using legacy parameters without considering specific load conditions (loading density, wrapping material type, presence of lumens) results in wet packs and repeated qualifications (Chen et al., 2024).

Lethality calculations assume an instantaneous inactivation effect of temperature. However, in the absence of moisture (dry heat due to an air lock), spore resistance increases by orders of magnitude, rendering the calculated lethality invalid (Cho & Chung, 2020).

The aim of this work is to develop a comprehensive risk-based model for the design and validation of steam sterilization cycles that ensures process robustness and full transparency for regulatory audit.

To achieve this aim, the following tasks are addressed:

1. To conduct an in-depth comparative analysis of thermodynamic profiles of successful and unsuccessful sterilization cycles based on real engineering data.
2. To identify the physical mechanisms underlying the formation of cold spots and barriers to steam penetration in complex mixed loads.
3. To adapt the FMEA methodology to the cycle development stage by linking each process parameter (heating rate, vacuum depth) to a specific failure mode.
4. To formulate practical acceptance criteria for engineering studies that exceed minimal regulatory requirements and thereby provide a margin of robustness (Design Space).

The study's scientific novelty lies in integrating quantitative data from high-precision thermal mapping with qualitative risk assessment tools during the pre-validation stage. For the first time, the correlation between equilibration dynamics (equilibration time) and the stability of the sterilization plateau is examined in detail as a predictor of PQ (Performance Qualification) success. The work proposes a shift from a static template to a dynamic risk object, which requires targeted engineering controls.

II. MATERIALS AND METHODS

The study was conducted within the paradigm of mixed-methods research, combining experimental data collection (engineering runs of the autoclave), quantitative analysis (statistical processing of thermograms), and qualitative synthesis (risk analysis and interpretation of regulatory requirements). Quality by Design (QbD) serves as the key methodological principle, according to which, in this context, sterility must be built into the process at the development stage rather than confirmed solely by end-product testing. The methodological logic is implemented through an iterative cycle including sequential stages of risk assessment, parameter design, empirical testing, data analysis, and optimization.

The primary data-collection tool was the wireless validation system, Ellab TrackSense Pro. Its use is justified by a combination of high measurement

accuracy (0.05°C), stable performance under vacuum and saturated steam, and the ability to place loggers inside sealed packages without compromising their integrity, which fundamentally distinguishes this approach from wired thermocouples. The configuration used an array of 18 temperature loggers (Rigid and Flexible sensors) and one integrated pressure/temperature sensor. The sampling frequency was 1 second, enabling registration of rapid transient processes characteristic of vacuum pulsations and equilibration stages.

The object of study comprised mixed loads typical for biopharmaceutical manufacturing. These included metal instruments, characterized by high thermal conductivity and low heat capacity; porous materials (e.g., gowns and filters), which exhibit heightened complexity in air removal; and items with cavities and tubing where there is a risk of air pocket formation. Additionally, containers with liquids were included, which possess pronounced thermal inertia and thus influence warming dynamics and temperature distribution throughout the cycle.

The analytical component encompassed thermodynamic analysis, lethality calculation, and risk analysis. Thermodynamic analysis was based on assessing deviations of the actual temperature from the theoretical temperature of saturated steam, calculated using the Antoine equation or steam tables derived from the measured pressure. Significant negative deviation was interpreted as evidence of air presence, since the partial pressure of air reduces the temperature of the steam–gas mixture, whereas positive deviation was considered a possible indication of superheated steam.

For lethality calculation, the standard cumulative lethality algorithm defined in ISO 17665 and pharmacopoeias was applied. To systematize cause–and–effect relationships and identify critical process vulnerabilities, a modified FMEA (Failure Mode and Effects Analysis) focused on sterilization cycle parameters was used. For each cycle stage, including air removal, heating, holding, and drying, potential failure modes, their causes, and detection methods were defined, including such scenarios as incomplete air removal from the center of a package.

III. RESULTS

This section presents a detailed analysis of the data obtained during the engineering studies. Comparing the failed and successful cycles provides a clear illustration of how process parameters influence the physics of sterilization.

Sensor placement strategy and identification of critical zones

The first stage of the study involved defining monitoring points. The effectiveness of validation directly depends on the ability to capture the worst-case scenario. Figure 1 visualizes the three-dimensional map of sensor placement.

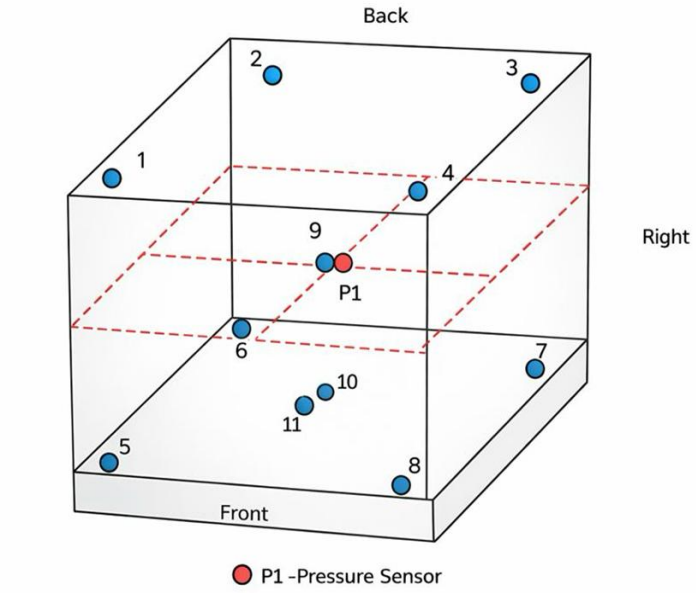


Fig. 1. Autoclave Logger placement diagram

The loggers, indicated by blue points LC 01–LC 18, are not distributed uniformly but are positioned deliberately according to a logic aimed at identifying zones with the highest probability of unfavorable heat and mass transfer conditions. This placement is oriented not toward formal symmetry but toward enhancing the measurement scheme's sensitivity to spatial temperature nonuniformity and to effects associated with residual air and condensation.

A portion of the sensors is installed at the geometric extrema of the chamber, i.e., in its corners and in the upper and lower regions. These positions serve as reference points because they reflect extreme conditions of heat distribution throughout the volume and allow detection of potential gradients arising

from specific features of steam circulation and the gravitational stratification of the gas mixture.

A separate group of loggers is placed in functional zones, primarily in the drain area. This region is interpreted as a critical point because condensate flows into it, and the coldest, heaviest gas-air mixture accumulates there. As a result, the drain zone is potentially associated with the formation of local cold regions and with the highest risk of failing to achieve target sterilization conditions.

The remaining sensors are placed directly inside the load, including the internal volumes of packages and cavities, in order to simulate the most difficult locations for steam penetration, designated as cold spots. Such positioning enables recording conditions in regions where steam access is hindered by geometry, material porosity, or the presence of cavities, thereby enabling experimental verification of the most critical scenarios for complete air removal and uniform heating.

A pressure sensor, P1, is placed at the center of the chamber. This configuration (18+1) exceeds the minimum requirements of many standards (typically 12 sensors for chambers up to 1 m^3), which allows a more detailed picture of the thermal field to be obtained (Boehler et al., 2021). Cold spots are rarely located where legacy templates suggest, so a dense sensor grid is necessary to empirically identify risk zones.

Analysis of the unsuccessful cycle (Failed Study Data)

The first iteration of cycle development was carried out using parameters based on standard templates, without thorough adaptation to the load specifics. Figure 2 shows classical signs of thermodynamic instability.

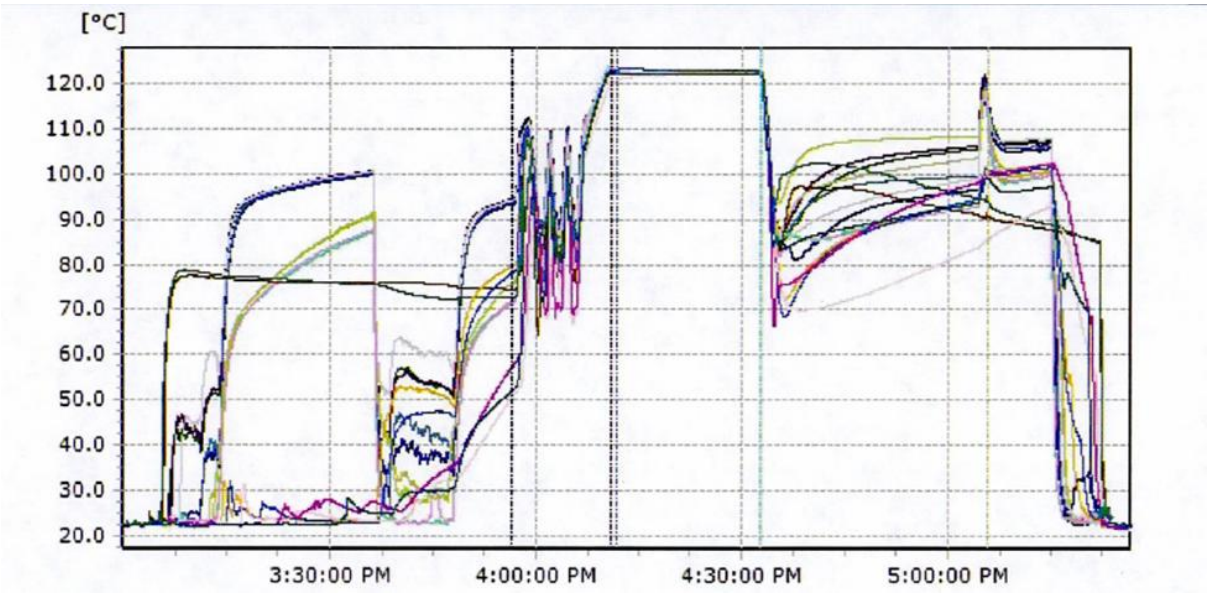


Fig. 2. Failed Study Data Autoclave Cycle Graph

The graph demonstrates classical indications of thermodynamic instability. During the come-up phase, the temperature curves of individual sensors do not form a single coherent bundle; instead, a pronounced divergence of lines and a significant spread between the hottest and coldest points are observed. This pattern indicates spatial nonuniformity in heat transfer conditions within the chamber and shows that different regions of the medium are heating at different rates.

Subsequently, slow equilibration is observed: some sensors reach the target temperature of 121°C with a marked delay relative to the control sensor positioned in the drain zone. In thermodynamic terms, this means that temperature-field equalization and the approach of the system to a quasi-stationary state do not occur synchronously throughout the volume but with substantial time lags at individual points.

During the sterilization plateau, instability persists. Temperature fluctuations are seen during the holding phase, indicating dynamic processes in the

steam–gas medium and at the load surface. Such fluctuations are interpreted as manifestations of air pockets that periodically compress and expand, or as resulting from steam condensation on relatively cold regions, which leads to local changes in the thermal regime.

Figure 3 confirms the use of 16 temperature sensors, but the absence of an independent pressure logger.

Sensor ID	Name	Validation Equipment	Description	Sample Rate 1/2 (hh:mm:ss)	Type	Sensor group
685941	LC 01	715757	Temperature	00:00:01 / N/A	LC	N/A
685943	LC 02	715739	Temperature	00:00:01 / N/A	LC	N/A
685946	LC 03	715889	Temperature	00:00:01 / N/A	LC	N/A
685948	LC 04	715753	Temperature	00:00:01 / N/A	LC	N/A
685950	LC 05	715913	Temperature	00:00:01 / N/A	LC	N/A
686043	LC 06	715892	Temperature	00:00:01 / N/A	LC	N/A
686044	LC 07	715914	Temperature	00:00:01 / N/A	LC	N/A
686045	LC 08	715920	Temperature	00:00:01 / N/A	LC	N/A
686046	LC 09	715887	Temperature	00:00:01 / N/A	LC	N/A
686049	LC 10	715882	Temperature	00:00:01 / N/A	LC	N/A
686050	LC 11	715915	Temperature	00:00:01 / N/A	LC	N/A
686052	LC 12	715905	Temperature	00:00:01 / N/A	LC	N/A
686056	LC 13	715903	Temperature	00:00:01 / N/A	LC	N/A
686057	LC 14	715902	Temperature	00:00:01 / N/A	LC	N/A
686063	LC 15	715765	Temperature	00:00:01 / N/A	LC	N/A
686067	LC 16	715780	Temperature	00:00:01 / N/A	LC	N/A

Fig. 3. 16 Ellab Data Logger used and No Pressure data logger

This is a critical study design error. Without independent pressure data, it is impossible to calculate the theoretical saturated steam temperature and thus to confirm the presence of saturation conditions.

The data in Figure 4 show catastrophic outcomes for a biopharmaceutical process.

Equilibration Time Statictics

Name:	Equilibration Time Statictics
Description:	Time difference from sterilization start (reference temp reaching 121C) and all remaining loggers reaching 121C.

Input parameters

Data Series Type:	Temperature
Data series:	LC 01, LC 02, LC 03, LC 04, LC 05, LC 06, LC 07, LC 08, LC 09, LC 10, LC 11, LC 12, LC 13, LC 14, LC 15, LC 16
Start Time:	4:10:46 PM
Stop Time:	4:10:51 PM
Output:	Global Data analysis
Functions:	Min., Max., Average

Global Data analysis

Min.	119.8 °C	4:10:46 PM	LC 14
Max.	123.9 °C	4:10:46 PM	LC 05
		4:10:47 PM	LC 05
		4:10:48 PM	LC 05
Average	122.9 °C		

Fig. 4. Equilibration Time (Failed Cycle)

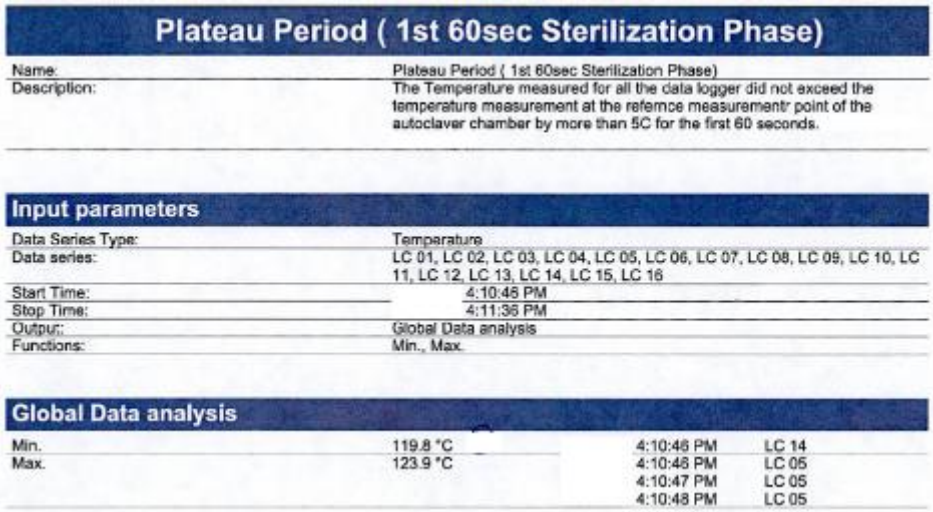
At the presumed equilibration, when the control sensor reached 121°C, the minimum temperature in the load was 119.8°C, as recorded by sensor LC 14.

Consequently, point LC 14 did not reach sterilization conditions. If a critical product component were located at this point, it would have remained non-sterile. The difference of 1.2°C may appear small. However, on a logarithmic scale of microbial inactivation, such a deviation results in a substantial reduction in lethality. Figure 5 illustrates the plateau period.

Throughout the plateau, the minimum temperature remained at 119.8°C. This demonstrates that the issue was not a transient heating lag but a systemic problem, most likely the presence of a stable air pocket acting as a thermal insulator, preventing steam from contacting the surface.

Analysis of the optimized cycle (Pass Data)

Based on the analysis of the failed cycle, modifications were introduced: parameters of vacuum pulsations were adjusted (increased depth and number), and the load configuration was altered to facilitate air removal. Figure 6 shows the autoclave cycle graph for the pass data.



Plateau Period (1st 60sec Sterilization Phase)

Name:	Plateau Period (1st 60sec Sterilization Phase)
Description:	The Temperature measured for all the data logger did not exceed the temperature measurement at the refernce measurement point of the autoclaver chamber by more than 5C for the first 60 seconds.

Input parameters

Data Series Type:	Temperature
Data series:	LC 01, LC 02, LC 03, LC 04, LC 05, LC 06, LC 07, LC 08, LC 09, LC 10, LC 11, LC 12, LC 13, LC 14, LC 15, LC 16
Start Time:	4:10:46 PM
Stop Time:	4:11:36 PM
Output:	Global Data analysis
Functions:	Min., Max.

Global Data analysis

Min.	119.8 °C	4:10:46 PM	LC 14
Max.	123.9 °C	4:10:48 PM	LC 05
		4:10:47 PM	LC 05
		4:10:48 PM	LC 05

Fig. 5. Plateau Period (Failed Cycle)

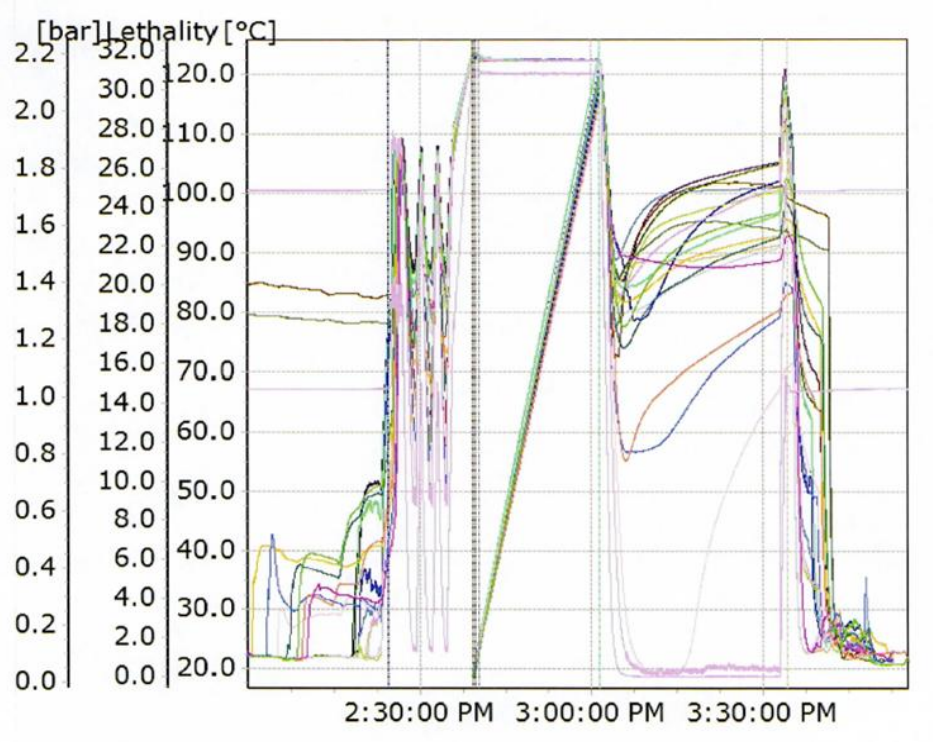


Fig. 6. Pass Data Autoclave Cycle Graph

Visually, the graph differs drastically from the previous one. Almost immediately after the start of the heating phase, convergence is observed: all 18 temperature curves merge into a narrow band, indicating coordinated behavior of measurement points and rapid transition of the system to a thermally similar state throughout the entire volume.

The cycle structure is clearly expressed on the

graph. Transitions between vacuum, heating, sterilization, and drying appear sharp and controlled, without extended segments and without indications of prolonged equilibration typical of unstable regimes. This compactness of the phase pattern reflects process controllability and the predictability of temperature dynamics.

During the holding phase, stability is maintained: the temperature of all sensors remains consistently within the range of 121–124°C. The absence of notable divergence between curves and the lack of pronounced oscillations at the plateau correspond to a state in which the temperature field remains homogeneous within the specified window and is maintained without significant disturbances. Figure 7 illustrates the use of 18 Ellab data loggers and one pressure data logger.

Sensor ID	Name	Validation Equipment	Description	Sample Rate 1/2 (hh:mm:ss)	Type	Sensor group
685941	LC 01	715757	Temperature	00:00:01 / N/A	LC	N/A
685943	LC 02	715739	Temperature	00:00:01 / N/A	LC	N/A
685946	LC 03	715889	Temperature	00:00:01 / N/A	LC	N/A
685948	LC 04	715753	Temperature	00:00:01 / N/A	LC	N/A
685950	LC 05	715913	Temperature	00:00:01 / N/A	LC	N/A
686043	LC 06	715892	Temperature	00:00:01 / N/A	LC	N/A
636557	LC 07	715909	Temperature	00:00:01 / N/A	LC	N/A
686045	LC 08	715920	Temperature	00:00:01 / N/A	LC	N/A
686046	LC 09	715887	Temperature	00:00:01 / N/A	LC	N/A
686049	LC 10	715882	Temperature	00:00:01 / N/A	LC	N/A
686050	LC 11	715915	Temperature	00:00:01 / N/A	LC	N/A
686052	LC 12	715905	Temperature	00:00:01 / N/A	LC	N/A
686056	LC 13	715903	Temperature	00:00:01 / N/A	LC	N/A
686057	LC 14	715902	Temperature	00:00:01 / N/A	LC	N/A
686063	LC 15	715765	Temperature	00:00:01 / N/A	LC	N/A
686067	LC 16	715780	Temperature	00:00:01 / N/A	LC	N/A
686069	LC 17	715799	Temperature	00:00:01 / N/A	LC	N/A
686070	LC 18	715747	Temperature	00:00:01 / N/A	LC	N/A
656186	LP 19	715899	Pressure	00:00:01 / N/A	LP	N/A
N/A	LP 19 - T-Theoretical 1	N/A	N/A	00:00:01 / N/A	TA	N/A

Fig. 7. 18 Ellab Data Logger used and 1 Pressure data logger

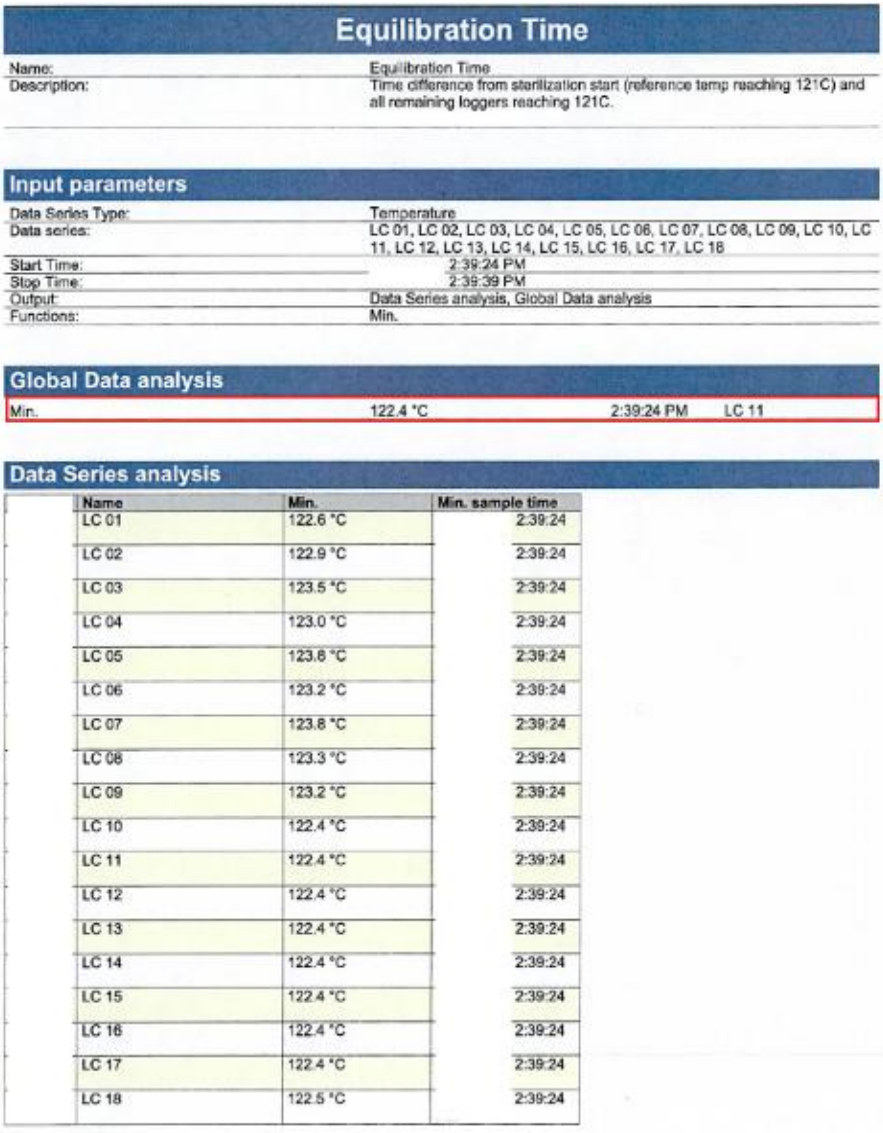
In the successful study, a pressure sensor (LP 19) was added. This allowed the report to include the line LP 19 – T-Theoretical, which shows the temperature corresponding to the current saturated steam pressure. The coincidence between actual temperatures and the theoretical curve confirms the steam quality (dryness >97%, absence of superheat). The equilibration time for the pass cycle is shown in Figure 8.

The equilibration time was less than 15 seconds. According to EN 285, the acceptable time for small loads lies within 15–30 seconds; however, the smaller this value, the better (BSI, 2021). Practically instantaneous equilibration is interpreted as an indicator of complete air removal, since the absence of delays between points indicates rapid establishment of saturated steam conditions throughout the volume.

The temperature corridor was achieved synchronously: all sensors entered the 121.0–124.0°C range simultaneously. Such simultaneous entry means that the temperature field was formed coherently, without pronounced local lags, and that the target window is maintained as a property of the system as a whole rather than a characteristic of isolated regions. Holding conditions after equilibration are shown in Figure 9.

In the summary analysis table for the holding phase, the minimum temperature was 122.2°C and was recorded by sensor LC 14, i.e., the same sensor that failed in the previous test. The maximum temperature reached 123.9°C and corresponded to sensor LC 05. This implies that the temperature distribution was very narrow, and cold spots were not strongly favored during the hold phase of the plateau.

From which follows that 1.2 degrees Celsius in excess of the 121 degree Celsius for each point will not exceed the upper limit of the safety temperature of 124 degrees Celsius, ensuring the effectiveness of the process while preserving the sterilized object in good conditions. Figure 10 shows how the lethality is calculated and what are the input values used.



Equilibration Time

Name:	Equilibration Time
Description:	Time difference from sterilization start (reference temp reaching 121C) and all remaining loggers reaching 121C.

Input parameters

Data Series Type:	Temperature
Data series:	LC 01, LC 02, LC 03, LC 04, LC 05, LC 06, LC 07, LC 08, LC 09, LC 10, LC 11, LC 12, LC 13, LC 14, LC 15, LC 16, LC 17, LC 18
Start Time:	2:39:24 PM
Stop Time:	2:39:39 PM
Output:	Data Series analysis, Global Data analysis
Functions:	Min.

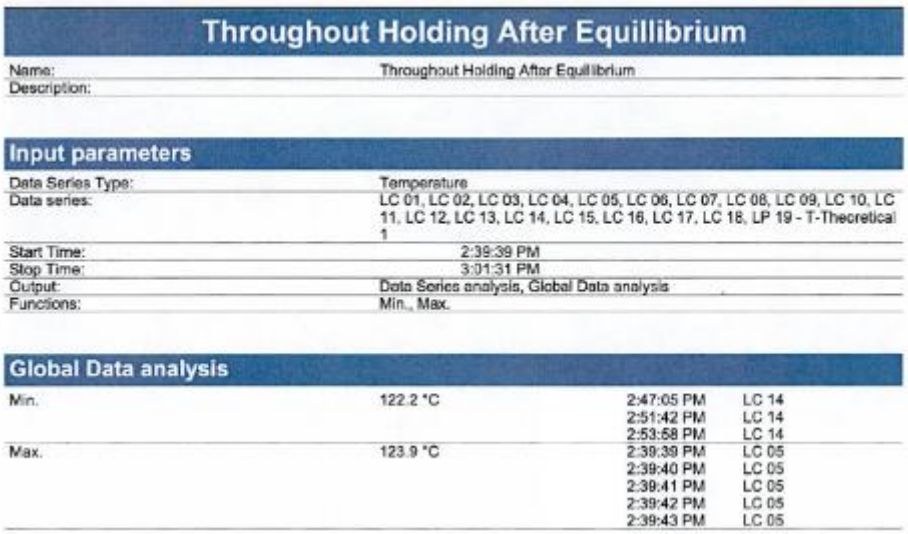
Global Data analysis

Min.	122.4 °C	2:39:24 PM	LC 11

Data Series analysis

Name	Min.	Min. sample time
LC 01	122.6 °C	2:39:24
LC 02	122.9 °C	2:39:24
LC 03	123.5 °C	2:39:24
LC 04	123.0 °C	2:39:24
LC 05	123.6 °C	2:39:24
LC 06	123.2 °C	2:39:24
LC 07	123.8 °C	2:39:24
LC 08	123.3 °C	2:39:24
LC 09	123.2 °C	2:39:24
LC 10	122.4 °C	2:39:24
LC 11	122.4 °C	2:39:24
LC 12	122.4 °C	2:39:24
LC 13	122.4 °C	2:39:24
LC 14	122.4 °C	2:39:24
LC 15	122.4 °C	2:39:24
LC 16	122.4 °C	2:39:24
LC 17	122.4 °C	2:39:24
LC 18	122.5 °C	2:39:24

Fig. 8. Equilibration Time (Pass Cycle)



Throughout Holding After Equillibrium

Name:	Throughout Holding After Equillibrium
Description:	

Input parameters

Data Series Type:	Temperature
Data series:	LC 01, LC 02, LC 03, LC 04, LC 05, LC 06, LC 07, LC 08, LC 09, LC 10, LC 11, LC 12, LC 13, LC 14, LC 15, LC 16, LC 17, LC 18, LP 19 - T-Theoretical 1
Start Time:	2:39:39 PM
Stop Time:	3:01:31 PM
Output:	Data Series analysis, Global Data analysis
Functions:	Min., Max.

Global Data analysis

Min.	122.2 °C	2:47:05 PM	LC 14
		2:51:42 PM	LC 14
		2:53:58 PM	LC 14
Max.	123.9 °C	2:39:39 PM	LC 05
		2:39:40 PM	LC 05
		2:39:41 PM	LC 05
		2:39:42 PM	LC 05
		2:39:43 PM	LC 05

Fig. 9. Throughout Holding After Equilibrium

Lethality Overview

Input parameter

Ref. Temperature:	121.1 °C
Min. Temperature :	91.1 °C
Z-Value:	10 °C
D-Value:	1

Sensor lethality overview

Name	Description	Start Time	End Time	Max. Value
LC 01 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	30.2
LC 02 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	30.8
LC 03 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	31.2
LC 04 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	30.7
LC 05 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	31.9
LC 06 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	30.8
LC 07 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	31.0
LC 08 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	31.0
LC 09 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	31.1
LC 10 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.5
LC 11 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.4
LC 12 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.6
LC 13 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	31.0
LC 14 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.3
LC 15 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.6
LC 16 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.8
LC 17 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	29.6
LC 18 (F0) Lethality 1	L-Calculation	2:39:24 PM	3:01:31 PM	30.7

Fig. 10. Lethality Calculation and Input Parameter Used

Theoritical Temperature on Drain Probe

Name:	Theoritical Temperature on Drain Probe
Description:	

Input parameters

Data Series Type:	Temperature
Data series:	LC 10, LC 11, LP 19 - T-Theoretical 1
Start Time:	2:39:24 PM
Stop Time:	3:01:31 PM
Output:	Data Series analysis, Global Data analysis
Functions:	Min., Max.

Global Data analysis

Min.	122.2 °C	2:53:58 PM	LC 11
Max.	122.6 °C	2:39:41 PM	LP 19 - T-Theoretica...

Data Series analysis

ID	Name	Min.	Min. sample time	Max.	Max. sample time
686049-1	LC 10	122.2 °C	2:47:05	122.5 °C	2:39:35
686050-1	LC 11	122.2 °C	2:53:58	122.5 °C	2:39:38
N/A	LP 19 - T-Theoretical 1	122.3 °C	2:51:42	122.6 °C	2:39:41

Fig. 11. Temperature at the Drain of the Autoclave

The lethality calculation demonstrates the process's integral effectiveness. The lethality range was 29–32 minutes, reflecting the accumulated sterilizing effect over the entire temperature–time profile rather than simply the attainment of a given temperature set point. In this representation, lethality serves as an aggregate measure that integrates cycle dynamics into a single indicator, allowing comparison across sensors and runs.

The values obtained exceed the standard overkill criterion by≥12 minutes, thereby providing an extremely high sterility assurance level of $<10^{-6}$. Notably, sensor LC 14, which demonstrated the lowest lethality (highlighted in red), still achieved the target values; this provides direct confirmation of cycle robustness, as even the weakest point in terms of integral effect remains within the required level. The temperature at the autoclave drain is shown in Figure 11.

The data show that the temperature in the drain (traditionally the coldest point) matched the temperature in the chamber. This eliminates the risk of a cold plug in the condensate removal system, which is often a root cause of failures (Failure Mode: Drain blockage). Figure 12 demonstrates that no temperature exceeds 124°C during the entire sterilization cycle.

Name:	No temperature exceed 124C during the entire cycle		
Description:			
Input parameters			
Data Series Type:	Temperature		
Data series:	LC 01, LC 02, LC 03, LC 04, LC 05, LC 06, LC 07, LC 08, LC 09, LC 10, LC 11, LC 12, LC 13, LC 14, LC 15, LC 16, LC 17, LC 18		
Start Time:	2:24:26 PM		
Stop Time:	3:34:18 PM		
Output:	Data Series analysis, Global Data analysis		
Functions:	Max.		
Global Data analysis			
Max.	123.9 °C	2:39:35 PM	LC 05
		2:39:37 PM	LC 05
		2:39:38 PM	LC 05
		2:39:39 PM	LC 05
		2:39:40 PM	LC 05
		2:39:41 PM	LC 05
		2:39:42 PM	LC 05
		2:39:43 PM	LC 05

Fig. 12. Temperature report during the entire sterilization cycle

The absence of overheating is evident. The maximum temperature was 123.9°C. This is critically important for preserving the physical properties of packaging materials and the product itself. Overheating may lead to embrittlement of plastics or deformation of stoppers (Zaborniak et al., 2024).

IV. DISCUSSION

This section synthesizes the empirical data obtained with theoretical concepts and regulatory requirements, forming an integrated picture of the risk-based approach.

Thermodynamics of failure: Why templates do not work

Analysis of the failed cycle (Section 3.2) provides a unique insight into sterilization physics. The fact that sensor LC 14 indicated 119.8°C while the autoclave control sensor showed 121°C is a classic manifestation of thermodynamic imbalance caused by an air pocket.

Air is an excellent thermal insulator. Its heat transfer coefficient is hundreds of times lower than that of condensing steam. Moreover, according to Dalton's law, the total pressure in the chamber is the sum of the partial pressures of steam and air (Tessarolo & Masè, 2025).

If air remains in a local region (for example, inside a tube or a dense package), the partial pressure of steam there is lower than in the rest of the chamber. Consequently, the saturation temperature at that point will also be lower. This explains why sensor LC 14 recorded a temperature lower than the setpoint.

A second-order insight is that, in the failed study, using only temperature sensors without pressure recording would essentially obscure the nature of the problem. In such a scenario, a reduced temperature would be observed, but its interpretation would

remain ambiguous: it would be impossible to determine whether the deviation was due to air in the steam–gas mixture or to condensation on cold surfaces.

The addition of a pressure sensor in the pass study fundamentally changes analytical reliability. The presence of measured pressure enables mathematical confirmation of saturated steam conditions, allowing saturation to be demonstrated not only through visual similarity of thermograms but also via calculation-based verification. Thus, the process physics is examined as the causal basis of the observed temperature regime, not merely the final result of reaching a temperature setpoint.

The role of FMEA in cycle design

The traditional approach treats validation as a confirmatory exercise, i.e., a procedure that verifies the conformity of an already-defined process to requirements. The approach proposed here, by contrast, uses FMEA as a design tool, emphasizing establishing process controllability at the development stage rather than post hoc confirmation of outcomes.

Instead of operating with abstract risks, the methodology advocates formulating engineering questions that convert uncertainty into testable hypotheses. For example, the risk of variable load density and its direct consequence, unpredictable cold-spot migration, is considered. In response, mitigation is embedded into the cycle design: the use of additional vacuum pulses is envisaged, and the equilibration time is introduced as a critical parameter that determines the start of the lethality calculation.

This FMEA adaptation enables a transition from passive observation to active control. In particular, in the pass cycle, rapid equilibration of less than 15 seconds is interpreted not as a fortuitous outcome but as the result of deliberate modification of vacuum parameters, justified through FMEA. In this sense, the cycle ceases to be a black box and becomes a transparent process in which causal relationships among settings, medium physics, and final performance indicators are subject to control.

Regulatory transformation: From Pass to Defendable

Modern regulatory requirements (ISO 17665-1:2024, Annex 1) shift the focus toward rationale. A cycle is considered validated only when the team can explain it without resorting to the report text.

ISO 17665-1:2024 requires a clear definition of the worst-case load (ISO, 2024). The graphical data presented indicate that the selection of measurement points and sensor locations, including the drain zone and package centers, was not random. Rather, it was based on an understanding of worst-case physics and an intention to record parameters precisely where the probability of deviations is highest.

EU GMP Annex 1 introduces the concept of a Contamination Control Strategy (CCS), in which autoclaving is considered a barrier (PMS, 2022). The successful cycle (Pass Data) with $F_0 \approx 30$ minutes demonstrates a substantial margin of robustness compared to the usual 12–15 minutes and can therefore serve as a strong CCS argument. This shows that the process can maintain effectiveness even in the face of an unforeseen increase in bioburden.

Critique and limitations of the lethality concept

It is important to recognize that lethality is the integral of temperature over time. This indicator accumulates lethality contributions even at 115°C because it mathematically accounts for each part of the temperature profile's contribution to the total effect. In this form, lethality is a convenient summary metric. However, it describes the integral thermal exposure but does not explain the physical nature of the medium in which it occurs.

If a temperature of 115°C is conditioned by the presence of dry air, the true biological inactivation will be negligible, since the z-value for dry heat is substantially higher than 10°C. Consequently, regimes with identical nominal lethality can exhibit fundamentally different biological performance depending on whether saturated steam is ensured or whether the process occurs in a steam–gas mixture containing air.

Therefore, acceptance criteria must be constructed hierarchically. First, strict temperature limits should be defined, including the requirement that Min ≥ 121.0°C, and equilibration time should be controlled as an indicator of complete air removal and formation of homogeneous conditions. Only after these fundamental physical conditions are met does it make sense to use lethality as the final integral indicator.

A practical rule of explainability

Validation is knowledge, not a report. If an operator or engineer cannot explain why four vacuum pulses are used instead of three, the process is at risk. A deep understanding of the physics demonstrated in the study (P/T correlation, drain analysis) builds operational resilience. In the event of future deviations (for example, vacuum pump failure), personnel will be able to diagnose the issue rapidly by understanding its root cause rather than simply following an instruction to restart the cycle.

V. CONCLUSION

The study convincingly demonstrates that the development of sterilization cycles in modern biopharmaceutical manufacturing requires a shift from empirical templates to a scientifically grounded risk-based approach. Under conditions of increasingly complex products and variable loads, process robustness can no longer rely solely on proven regimes and formal adherence to setpoints, because understanding the causal relationships among heat and mass transfer physics, cycle architecture, and actual inactivation performance becomes decisive.

The data obtained indicate pronounced physical non-uniformity of the process. Mixed and porous loads generate complex thermodynamic conditions in which standard parameters do not ensure reliable air removal and therefore do not guarantee homogeneous attainment of sterilizing conditions at all points. The results of the failed study confirm the existence of local risk zones even when the autoclave controller displays normal values, i.e., when the system-level signal masks critical local deviations.

Against this background, the effectiveness of engineering metrics that are more sensitive to the actual process physics than a simple extension of holding time is demonstrated. In particular, equilibration time and pressure–temperature correlation emerge as more reliable indicators of success, as they provide information on the completeness of deaeration and the attainment of saturated steam conditions. In the successful cycle, an equilibration time of less than 15 seconds proved a practical marker of effective air removal and rapid temperature-field equalization.

The role of FMEA as a design tool is emphasized separately. Integrating risk analysis at the cycle design stage enables proactive elimination of failure causes, such as air pocket formation, through targeted modifications to cycle parameters (e.g., vacuum pulses), rather than through trial-and-error iterations. In this way, risk is moved from the realm of post hoc detection into the realm of controlled engineering decision-making.

Finally, the proposed approach demonstrates alignment with new standards and regulatory logic. It is harmonized with ISO 17665-1:2024 and EU GMP Annex 1 requirements, ensuring not only product sterility but also the defendability of the process before auditors, grounded in transparent criteria, reproducible metrics, and traceable justifications for decisions.

The practical significance of the work lies in providing a ready-to-use framework for validation engineers that enables greater reliability in sterilization processes, reduced deviations, and the highest level of patient safety. This framework is oriented toward reproducibility, diagnostic capability, and controllability, making it applicable under conditions of manufacturing variability and rising regulatory expectations.

LIST OF ABBREVIATIONS

BSI - British Standards Institution

CCS - Contamination Control Strategy

EMA - European Medicines Agency

EN - European Norm

EU GMP - European Union Good Manufacturing Practice

FDA - Food and Drug Administration

FMEA - Failure Mode and Effects Analysis

ISO - International Organization for Standardization

LC - Temperature logger/channel ID (e.g., LC01–LC18)

LP - Pressure logger (e.g., LP19)

NCGs - Non-Condensable Gases

P/T - Pressure/Temperature correlation

PQ - Performance Qualification

QbD - Quality by Design

QRM - Quality Risk Management

SAL - Sterility Assurance Level

VI. AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analysed during the current study are not publicly available due to confidentiality and GMP documentation restrictions, but are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The author declares that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

The author declares that they solely conceived and designed the study, performed the data collection, conducted the analysis and interpretation, and wrote the final manuscript.

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