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Risk-Based Qualification of Dual-Use Refrigerated Incubators Using Temperature Bracketing and Lifecycle Verification

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Abstract *— The article examines risk-based qualification of dual-use refrigerated incubators using temperature bracketing and verification embedded into equipment lifecycle management. The relevance of the work stems from the need to simultaneously satisfy increasingly stringent GxP regulatory expectations and the demand for operational leanness in laboratory infrastructure, where the traditional V-model of validation results in an excessive volume of mapping activities. The objective of the study is to develop a scientifically justified qualification scheme that, with a minimal number of tests, enables reliable extrapolation of results between boundary and intermediate setpoints. The novelty of the approach lies in integrating engineering data on the thermal behavior of the incubator with the ASTM E2500 methodology and quality risk management principles, as well as in introducing the concept of dynamic qualification, which treats recovery rate as a critical quality attribute. Based on mapping at +5.0 °C and +57.5 °C using 15 calibrated data loggers, a performance envelope is established within which intermediate modes can reasonably be regarded as qualified; acceptance criteria, sensor placement schemes, and the logic of risk-based monitoring within the lifecycle framework are substantiated. The article is intended for validation and quality specialists, equipment engineers, authors of GxP laboratory procedures, and regulatory inspectors.*

Keywords *— **Equipment Qualification, Temperature Mapping, GxP, Risk-based Approach, Refrigerated Incubators, Temperature Bracketing.***

I. INTRODUCTION

In the context of the globalization of the pharmaceutical market and tightening requirements for medicinal product quality, the reliability of laboratory equipment has shifted from a purely technical concern to a strategic risk [1]. According to the guidelines of the International Council for Harmonisation, in particular ICH Q9 Quality Risk Management, manufacturers are required to demonstrate not merely the completion of testing but a mechanistic understanding of equipment behavior [2].

Dual-use refrigerated incubators occupy a unique niche. Unlike standard refrigerators, these devices must provide precise control over a wide dynamic temperature range. This creates a complex engineering challenge: the control system must switch efficiently between heating and cooling modes, avoiding hysteresis and temperature oscillations, especially in regions near ambient temperature.

The traditional validation paradigm, which has dominated the industry for decades, has assumed extensive testing: temperature mapping was carried out at all intended operating setpoints. This highly resource-intensive approach frequently failed to reveal latent defects that manifest only under extreme loads [3]. With the advent of ASTM E2500 and the revised Annex 15 to the EU GMP Guide, the focus

shifted toward verification, a continuous process of confirming system fitness for use, grounded in scientific justification and assessment of critical aspects [4, 5].

The core problem considered in this work lies in the tension between the need to ensure absolute data integrity in dual-use systems and the drive for operational efficiency. Validation engineers face a dilemma: how to demonstrate that an incubator tested at 5 °C and 57.5 °C will operate reliably at 25 °C without performing physical mapping at the intermediate point.

The absence of clear methodological guidance on the application of bracketing and matrixing strategies to small-volume laboratory equipment, as opposed to industrial warehouses or autoclaves, creates uncertainty [6]. This regulatory risk is not merely theoretical: a 2026 analysis of FDA Form 483 observations for biologics reported 29,173 biologics-related inspections, 1,084 Form 483s, and 630 warning letters between 2010 and 2025, with the most frequent cited deficiencies involving failure to follow written SOPs, inadequate contemporaneous documentation, and insufficient investigation controls [7].

The objective of the present study is to develop a scientifically justified methodology for the qualification of dual-use refrigerated incubators that harmonizes GxP requirements with lean methodology principles.

To achieve this objective, the following tasks were set:

1. To examine the physical principles of temperature regulation in dual-loop systems and their impact on the validation strategy.

2. To justify the application of temperature bracketing on the basis of risk analysis and thermodynamic models.

3. Using a real case study to analyze temperature profiles in the empty chamber, the loaded chamber, and the recovery after door opening modes.

4. To formulate practical guidance on sensor placement, selection of acceptance criteria, and lifecycle-based equipment management.

The scientific novelty of the work lies in integrating engineering data on the behavior of thermoelectric and compressor-based systems with the ASTM E2500 qualification methodology. The issue of validating dead zones of control when crossing the equilibrium point with the ambient environment is examined in detail, and a method is proposed for demonstrating stability through testing at boundary conditions. The concept of dynamic qualification is also introduced, in which the recovery rate is treated as a critical quality attribute.

II. MATERIALS AND METHODS

While IQ/OQ/PQ qualification is based on Engineering Good Practice, the process described in this study is based on the ASTM E2500 Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment, which utilizes subject-matter expert knowledge, QRM principles throughout the lifecycle, and vendor documentation to minimize duplicative testing. Although ASTM E2500 was developed for pharmaceutical manufacturing systems, its verification principles - including risk-based justification, use of subject-matter expert knowledge, and lifecycle-embedded confirmation of fitness for use - are applicable to GxP laboratory equipment supporting manufacturing activities, consistent with ICH Q9 risk management principles. A comparative analysis method was employed to contrast the requirements of different regulatory authorities, and a case study method was used to analyze specific validation data sets.

The object of analysis is a dual-use refrigerated incubator designed to operate in the range from +2 °C to +57.5 °C.

The equipment evaluated in this study was a refrigerated incubator, model 7001-33-1, serial number 7001-33-1-471P. The chamber volume was approximately 33 ft^3. The unit was qualified for operational use across a range extending from 2 °C to 60 °C. Within this range, the system was intended for use both as a refrigerator in the 2–8 °C range and as an incubator at elevated setpoints up to about 60 °C. The chamber was designed to maintain a uniform internal temperature field. The available system documentation and the reported chamber uniformity of 0.3 °C are consistent with a fan-driven air

circulation pattern typical of forced-air convection incubators.

Technical features of the system include forced air convection, a microprocessor-based PID controller, and the absence of active humidity control, which obviates the need for relative humidity mapping.

Data collection used a system of 15 independent temperature data loggers registered with the meteorological registry and calibrated to NIST or equivalent standards.

The mapping instrumentation consisted of individually identified probe designations recorded in the qualification package. Data acquisition during the mapping studies was performed at 5-minute intervals over 24-hour monitoring periods. Calibration status for all loggers remained current throughout the qualification window. A post-use three-point verification at 0 °C, 30 °C, and 65 °C confirmed that all 15 loggers remained within ±0.5 °C of the reference standard after completion of the mapping studies. The incubator display resolution was 0.1 °C according to the equipment specification. Traceability of the measurement system was supported by attached calibration certificates and post-use verification reports.

Temperature accuracy was controlled through the qualification program by the use of calibrated loggers with documented traceability and by post-study verification against a reference standard. Relative humidity was recorded as part of the environmental conditions during installation qualification, although active humidity control was not a functional attribute of the incubator and no RH mapping was required for chamber qualification.

The logger placement scheme is shown in Figure 1.

Ambient laboratory conditions were recorded as part of installation qualification. At the time of environmental verification, the laboratory temperature was approximately 20.0 °C and the relative humidity was 43.9%. These values were within the specified environmental range of 18–25 °C for equipment operation and qualification activities.

According to the placement scheme, sensors were installed as follows. Thirteen mapping sensors were distributed across the usable chamber space at five shelf levels to characterize the three-dimensional temperature field under both empty and loaded conditions. The arrangement included positions at the upper, middle, and lower regions of the chamber, with coverage of the front, rear, left, right, and central locations to detect local gradients associated with wall proximity, door effects, and airflow distribution. In addition to these mapping positions, sensor 14 was placed adjacent to the routine monitoring system probe on the middle shelf at the chamber center, and sensor 15 was placed adjacent to the incubator's control probe. This layout allowed direct comparison between the mapped chamber temperatures, the independent monitoring point, and the control point used by the equipment.

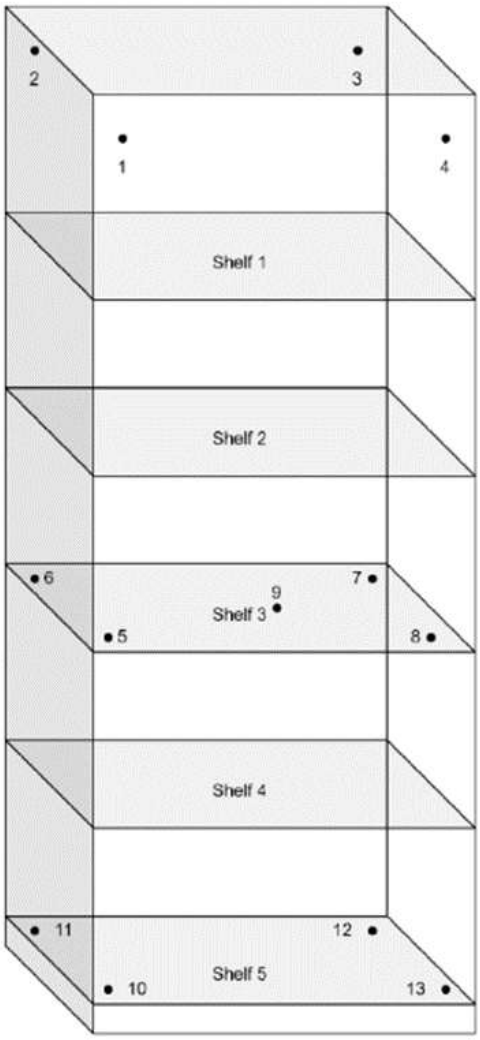

Fig.1. Logger Placement of Refrigerated Incubator

The placement configuration was documented in the qualification package through a sensor placement diagram, a table linking sensor identifiers to chamber locations, and annotated photographs of both the empty and loaded chambers. The protocol required

verification that sensors 1 through 13 were placed in accordance with the approved figure and that sensors 14 and 15 were positioned next to the monitoring and control probes. This 15-sensor arrangement provided dense spatial coverage for a chamber of this size and supported reconstruction of the thermal field during steady-state mapping and recovery studies [8].

The study employed a temperature bracketing strategy, which involves testing only the boundary values of the operating range. Predefined acceptance criteria were established for each qualification study and were linked to the qualification strategy for the boundary setpoints. For the 5.0 °C studies, all mapped temperatures in both empty and loaded chamber conditions were required to remain within 3.0 °C to 7.0 °C. For the 57.5 °C studies, all mapped temperatures were required to remain within 55.5 °C to 59.5 °C. These criteria corresponded to a ±2.0 °C band around each target setpoint. The selected limits were narrower than the broader operating spans assigned to intermediate routine setpoints in the user requirements and were used to challenge chamber control at the lowest and highest qualified temperatures. The pass or fail basis for each study was defined in the protocol before execution, and each executed section recorded whether the corresponding criterion was met.

The qualification logic underlying these limits was that successful control at the lower and upper boundaries of the qualified range under a tighter acceptance band would provide evidence that intermediate setpoints with wider allowable spans could be supported within the same control envelope. Separate acceptance criteria were also defined for functional tests, including alarm operation and recovery following door opening. For the Open Door / Recovery Test, recovery rate was predefined as a critical quality attribute and was considered acceptable when all mapped chamber locations returned to the applicable operating temperature band within 30 minutes after door closure following a standardized door-open challenge, with no sustained secondary excursion during the post-recovery observation period.

The selection of these points is based on the premise that system limitations are most pronounced at the range boundaries and therefore define worst-case conditions for assessing system performance. The +5.0 °C setpoint is considered the lower boundary and, at the same time, the worst case for the cooling system. In this mode, the risks of evaporator icing, stratification of cold air in the lower part of the chamber, and condensate formation are at their highest. Furthermore, this is a critical point for verifying freeze protection, since excursions below +2 °C are unacceptable.

The +57.5 °C setpoint corresponds to the upper boundary and represents the worst case for the heating system and thermal insulation. At this temperature, heat losses through walls and door seals reach a maximum, increasing the likelihood of temperature gradients and the appearance of cold spots at the periphery.

Intermediate points, such as 25 °C, 30 °C, or 37 °C, lie within this performance envelope and are subject to lower thermal stress. Consequently, successful validation at the boundaries of the range is considered evidence of system fitness for temperatures within the range.

The executed qualification protocol did not include replicate mapping runs at the same setpoint. Each mapped condition was performed once as defined by the approved strategy. The empty chamber and loaded chamber studies were executed at 5.0 °C and 57.5 °C, and no additional repeat runs were planned for those conditions within the scope of the initial qualification. Intermediate setpoints listed in the qualification rationale, including 22.5 °C, 25.0 °C, 28.0 °C, 30.0 °C, and 32.5 °C, were not mapped as separate empirical studies. Their qualification status was supported by the boundary-point verification strategy and by the tighter acceptance limits applied at the two extreme tested temperatures. A single 24-hour monitoring period at each setpoint was deemed sufficient based on the following rationale:

(1) PID-controlled systems at steady thermal setpoints exhibit low run-to-run variability once equilibrium is established;

(2) the 24-hour duration captures full diurnal laboratory temperature cycles; and

(3) this approach is consistent with WHO guidance on temperature mapping of cold-chain equipment [8] and with risk-based qualification principles under ASTM E2500. Replication was therefore not required within the scope of the initial qualification program.

The study included three test types for each boundary temperature. The Empty Chamber Temperature Distribution test consisted of mapping an empty chamber over 24 hours and was used to assess baseline temperature stability and field uniformity. The Loaded Chamber Temperature Distribution test was performed under a simulated full-load condition intended to represent the maximum routine storage configuration of the chamber. The Loaded Chamber Temperature Distribution test was performed under a simulated full-load condition intended to represent the maximum routine storage configuration of the chamber. The simulated load consisted of non-heat-generating storage materials distributed across all five shelf levels, with items positioned at the front, center, and rear regions of the chamber to challenge airflow distribution and thermal recovery under representative maximum-use conditions. The loading arrangement was documented through annotated chamber photographs retained in the qualification package. Because the executed protocol did not record the exact material type, dimensions, or total load mass, the loaded study was interpreted as a representative maximum-use challenge rather than as a quantitatively reproducible mass-based worst-case load.

This loading concept was used to evaluate chamber performance under conditions in which airflow pathways may be restricted by stored materials and in which added thermal mass may alter chamber response during transients. The qualification strategy treated the loaded condition as a representation of maximum routine use, with shelf utilization across the chamber volume rather than isolated loading at a single level.

The Open Door / Recovery Test was a dynamic assessment in which the door was opened for 30–60 seconds, after which the time required for the temperature to return to the specified band was recorded. Statistical data processing included calculation of minimum, maximum, and mean values, as well as estimation of process capability indices Cp and Cpk where applicable.

For each mapping study, the qualification record captured summary values that included the minimum observed temperature, the maximum observed temperature, the corresponding sensor locations, and the average temperature recorded at the monitoring probe over the 24-hour study period. Complete time-series outputs and channel statistics for all sensors were retained as attached printouts from the data acquisition software.. These attached records contained the detailed channel-level data needed to review minimum, maximum, and average values for each sensor across the full study interval. Under steady-state empty and loaded mapping conditions, all sensors remained within the predefined acceptance bands. Time out of range was limited to the intentional open-door challenge studies.

III. RESULTS

This section presents the analysis of empirical data obtained. Results are interpreted in light of compliance with GxP criteria.

3.1. Cooling Mode: +5.0 °C

The +5 °C mode is critical for the storage of thermolabile medicinal products, vaccines, and biological samples. The primary risk is deviation outside the 2–8 °C range, especially toward freezing. Figure 2 shows the Empty Chamber Study at 5 °C.

The graph indicates high system stability under the test conditions. All 15 sensors show readings within a narrow band from 5.0 °C to 5.4 °C. Over time, the trend lines appear almost flat: only minimal cyclic fluctuations are observed, typical of correctly tuned PID controllers or inverter compressors that maintain temperature without abrupt on/off transitions. Spatially, the system likewise demonstrates pronounced uniformity: the difference between the coldest and warmest sensors is less than 0.5 °C, which is markedly better than standard requirements of ±1 °C or ±2 °C.

A local feature is observed: one of the sensors records a temperature of about 6.0 °C. This value remains acceptable because it lies within the 2–8 °C specification, but it indicates a zone of heat ingress. Table data for the Empty Chamber Study at 5 °C are shown in Figure 3.

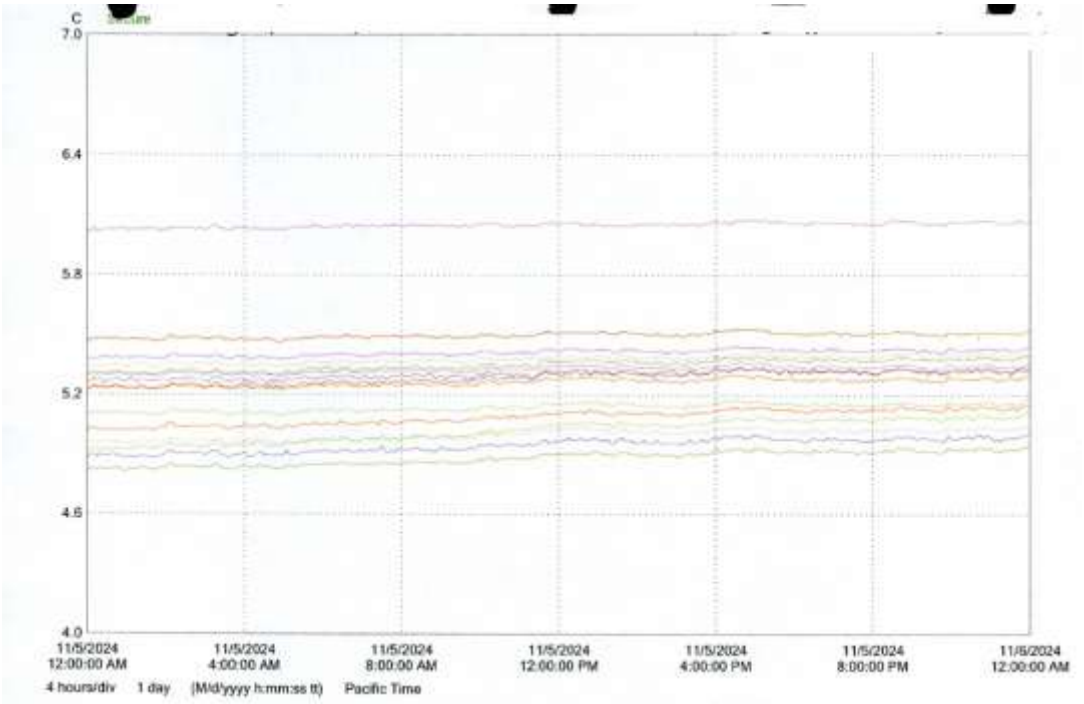

Fig.2. Empty Chamber Study Graph at 5°C

LN	Serial #	Maximum	Average	Minimum	Units	CH description
1	20221009	5.15	5.09	5.01	C	Sensor 1
2	20221009	4.94	4.88	4.81	C	Sensor 2
3	20221009	5.34	5.30	5.24	C	Sensor 3
4	20221009	6.08	6.05	6.01	C	Sensor 4
5	17271010	5.34	5.29	5.22	C	Sensor 5
6	17271010	5.11	5.02	4.91	C	Sensor 6
7	17271010	5.42	5.38	5.33	C	Sensor 7
8	17271010	5.45	5.41	5.37	C	Sensor 8
9	20251043	5.30	5.26	5.22	C	Sensor 9
10	20251043	5.40	5.35	5.29	C	Sensor 10
11	20251043	5.00	4.95	4.88	C	Sensor 11
12	20251043	5.36	5.33	5.29	C	Sensor 12
13	20251023	5.53	5.50	5.46	C	Sensor 13
14	20251023	5.18	5.14	5.09	C	Sensor 14
15	20251023	5.05	5.00	4.94	C	Sensor 15

Fig.3. Empty Chamber Study data at 5°C

This figure confirms the visual assessment: mean values lie between 5.0 °C and 5.4 °C, maxima are about 5.45 °C with a local peak at 6.0 °C, and minima reach approximately 4.9 °C. The executed qualification record for the 5.0 °C empty chamber study documented a minimum observed temperature of 4.81 °C at sensor 4 and a maximum observed temperature of 6.08 °C at sensor 2. The average temperature recorded at the routine monitoring probe was approximately 5.14 °C across the 24-hour interval. These values satisfied the predefined acceptance band of 3.0 °C to 7.0 °C for the low-temperature qualification study.

For the 5.0 °C loaded chamber study, the executed record documented a minimum observed temperature of 4.69 °C and a maximum observed temperature of 6.12 °C. All mapped channels remained within the study acceptance band of 3.0 °C

to 7.0 °C. These results supported the conclusion that the chamber remained within the required control region under simulated full-load conditions.

The absence of values below 2 °C indicates that conditions that lead to freezing risk do not occur, which is fundamentally important for freeze-sensitive products, e.g., certain vaccines.

The impact of loading on the temperature field is now considered. Figure 4 shows the Loaded Chamber Study graph at 5 °C.

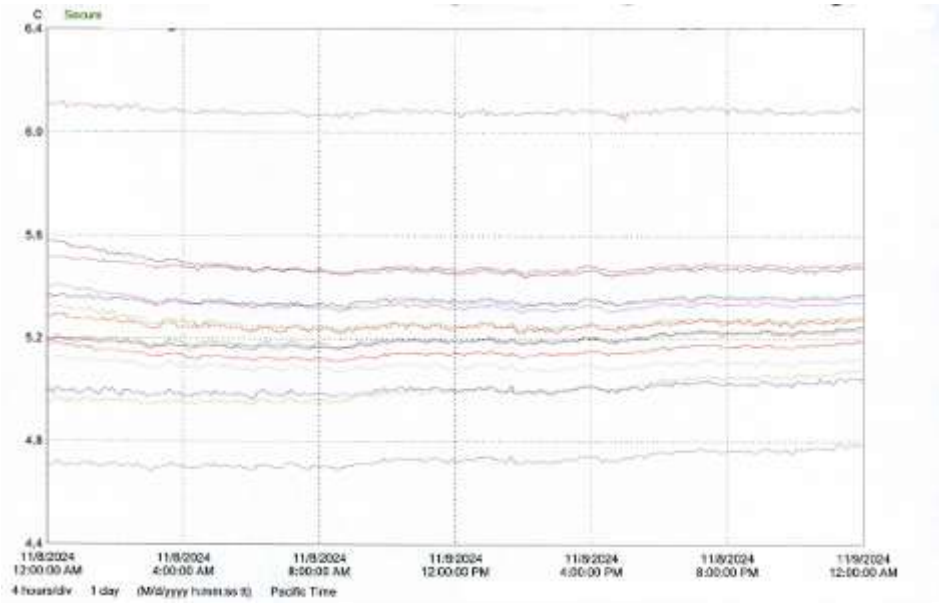

Fig.4. Loaded Chamber Study graph at 5 °C

Introduction of a load changes the dynamics. The temperature range expands to 4.4–6.4 °C. This expansion is associated with the load, creating aerodynamic resistance and slowing air mixing. Denser cold air settled toward the lower shelves, where Sensor [X] recorded a minimum of 4.4 °C. Warmer air accumulated near the upper door-adjacent region, where Sensor [Y] reached 6.4 °C. This vertical gradient of approximately 2.0 °C is consistent with buoyancy-driven stratification in fan-assisted refrigerated chambers under partial airflow obstruction.

Despite the broader range, temporal stability remains smooth: fluctuations are not abrupt because the load's thermal mass attenuates short-term variations. The Loaded Chamber Study data at 5 °C are presented in Figure 5.

LN	CH	Value	Maximum	Average	Minimum	Units	CH description
1	1		5.15	5.09	5.01	C	Sensor 1
2	2		4.94	4.88	4.81	C	Sensor 2
3	3		5.34	5.30	5.24	C	Sensor 3
4	4		6.08	6.05	6.01	C	Sensor 4
5	1		5.34	5.29	5.22	C	Sensor 5
6	2		5.11	5.02	4.91	C	Sensor 6
7	3		5.42	5.38	5.33	C	Sensor 7
8	4		5.45	5.41	5.37	C	Sensor 8
9	1		5.30	5.26	5.22	C	Sensor 9
10	2		5.40	5.35	5.29	C	Sensor 10
11	3		5.00	4.95	4.88	C	Sensor 11
12	4		5.36	5.33	5.29	C	Sensor 12
13	1		5.53	5.50	5.46	C	Sensor 13
14	2		5.18	5.14	5.09	C	Sensor 14
15	3		5.05	5.00	4.94	C	Sensor 15

Fig.5. Loaded Chamber Study data at 5 °C

This figure shows that the system maintains temperature within 2–8 °C with a substantial safety margin, confirming the effectiveness of forced convection even under loaded conditions.

The dynamic test associated with door opening is now examined. The open door graph for the loaded chamber mode at 5 °C is shown in Figure 6.

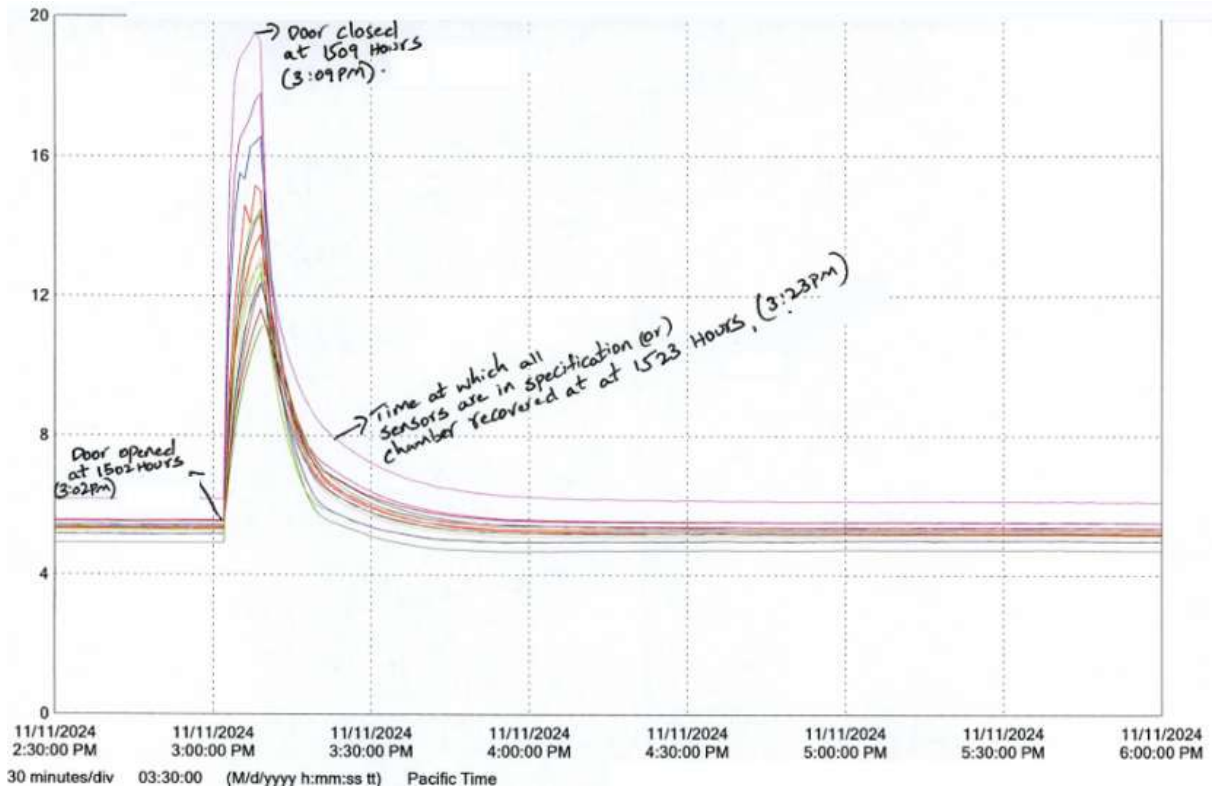

Fig.6. Open door graph study data at 5°C- Loaded Chamber

The graph reflects the system's response to a shock disturbance. At the moment of door opening, around 15:02, a sharp increase in temperature is recorded. Sensors located at the front register peaks up to 19 °C, while sensors deeper in the chamber respond less strongly, reaching about 12 °C. This non-uniformity corresponds to the mechanism of warmer room air entering through the door opening, which first affects the front zones, while more distant volumes are involved to a lesser extent.

During the 5.0 °C open-door challenge, the chamber was opened until the local display indicated departure from the 2–8 °C operating range. The out-of-range condition was triggered at 3:03 PM, and the door was closed at 3:09 PM, giving an open-door period of about 7 minutes. Following door closure, all sensors returned to the 2–8 °C range by 3:23 PM. The recovery interval after door closure was about 14 minutes. The maximum total excursion time from the first out-of-spec indication to the last sensor returning to range was recorded as 20 minutes. Data logging continued for 2 hours after recovery to document the return to steady operation. The Open Door logger data at 5 °C for the loaded chamber mode are shown in Figure 7.

LN	CH	Value	Maximum	Average	Minimum	Units	CH description
1	1		15.15	5.76	5.16	C	Sensor 1
2	2		14.46	5.22	4.66	C	Sensor 2
3	3		16.56	6.00	5.34	C	Sensor 3
4	4		19.53	6.89	6.10	C	Sensor 4
5	1		14.33	5.69	5.18	C	Sensor 5
6	2		12.94	5.42	4.94	C	Sensor 6
7	3		13.65	5.86	5.35	C	Sensor 7
8	4		17.79	6.18	5.50	C	Sensor 8
9	1		13.76	5.77	5.25	C	Sensor 9
10	2		11.16	5.61	5.18	C	Sensor 10
11	3		12.35	5.40	4.92	C	Sensor 11
12	4		12.33	5.78	5.36	C	Sensor 12
13	1		11.62	5.91	5.50	C	Sensor 13
14	2		12.68	5.76	5.28	C	Sensor 14
15	3		13.09	5.57	5.08	C	Sensor 15

Fig.7. Open door logger data study at 5 °C- Loaded Chamber

The table indicates that the short-term excursion has a substantial amplitude but remains limited in duration. For most products, such a spike is typically not critical, since, due to thermal inertia, the product temperature changes significantly less than the air temperature. At the same time, the recovery time of about 14 minutes has practical implications for drafting SOPs: the door should not be opened too frequently, and the interval between openings should be roughly 20–30 minutes to avoid cumulative heating.

3.2. Heating Mode: +57.5 °C

The high-temperature mode is used for accelerated stability studies, cultivation of thermophilic cultures, and viral inactivation. Here, the critical parameters are uniformity, the absence of localized overheating, and the accuracy of setpoint maintenance.

Stability and uniformity in the empty chamber are considered first. The Empty Chamber Study graph at 57.5 °C is shown in Figure 8.

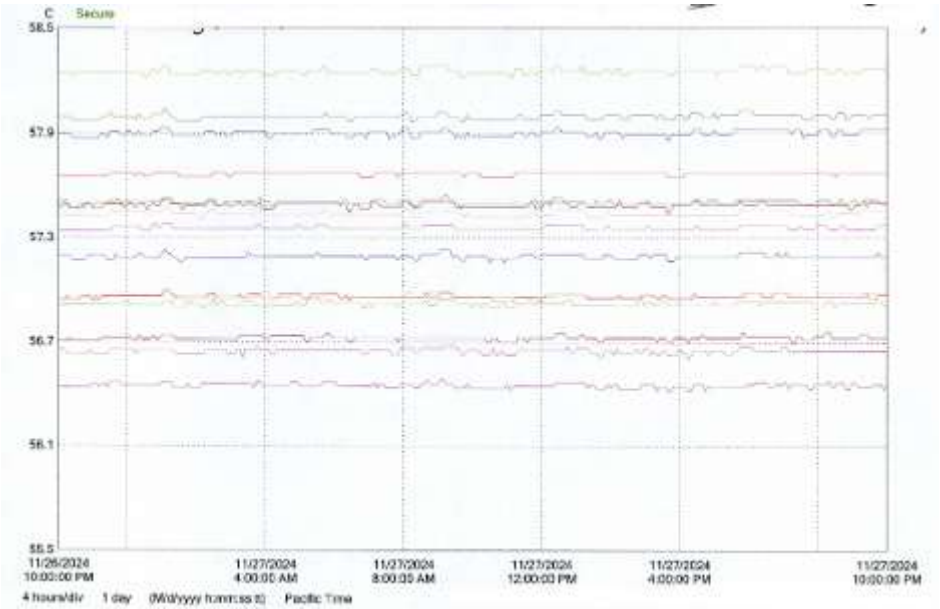

Fig.8. Empty Chamber Study graph at 57.5 °C

The graph reflects exceptional PID control stability. All sensors remain within a narrow 56.5–58.1 °C range, and deviations from the 57.5 °C setpoint are less than ±1 °C. The absence of pronounced sawtooth

patterns indicates that the proportional–integral–derivative, PID, parameters are optimally tuned, preventing overshoot when heaters are activated. The Empty Chamber Study data at 57.5 °C are given in Figure 9.

LN	CH	Value	Maximum	Average	Minimum	Units	CH description
1	1		57.00	56.96	56.93	C	Sensor 1
2	2		58.04	57.99	57.97	C	Sensor 2
3	3		57.23	57.19	57.15	C	Sensor 3
4	4		56.69	56.65	56.60	C	Sensor 4
5	1		57.52	57.49	57.44	C	Sensor 5
6	2		58.28	58.25	58.22	C	Sensor 6
7	3		56.98	56.97	56.95	C	Sensor 7
8	4		56.48	56.45	56.42	C	Sensor 8
9	1		57.67	57.67	57.65	C	Sensor 9
10	2		57.54	57.50	57.47	C	Sensor 10
11	3		57.93	57.90	57.86	C	Sensor 11
12	4		57.37	57.36	57.34	C	Sensor 12
13	1		56.76	56.72	56.70	C	Sensor 13
14	2		56.96	56.92	56.88	C	Sensor 14
15	3		57.47	57.44	57.41	C	Sensor 15

Fig.9. Empty Chamber Study data at 57.5 °C

The figure values confirm the high uniformity of the temperature field: spatial spread is minimal. This aligns with the conclusion that the cabinet insulation is of good quality, since even at a temperature difference ΔT of about 35 °C relative to the environment, the incubator walls do not create pronounced cold zones. Temperature recovery in heating mode is then considered. The Open Door Study graph at 57.5 °C is presented in Figure 10.

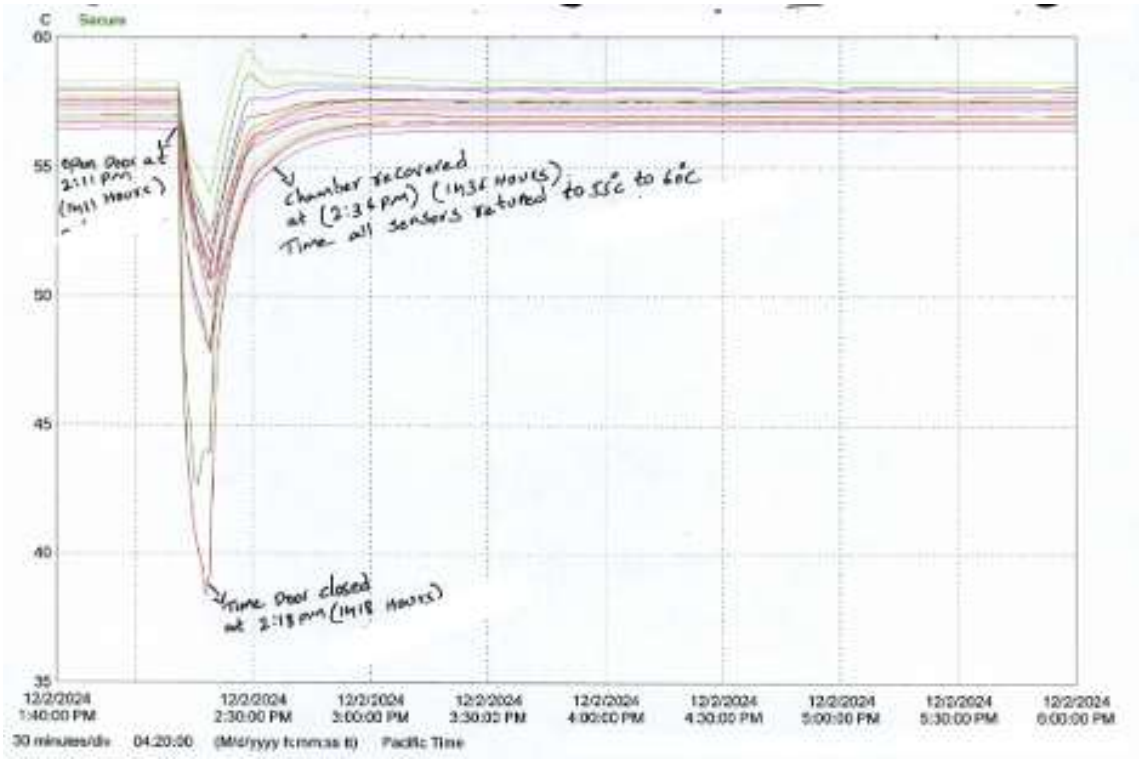

Fig.10. Open Door Study with Loaded Chamber graph at 57.5 °C

The graph illustrates a situation opposite to cooling: when the door is opened, a sharp drop in

temperature is observed. Minimum values reach 38–45 °C, reflecting the influence of cooler laboratory air at approximately 22 °C. After the door is closed, recovery begins, and return to the setpoint takes about 17 minutes, from around 14:18 to 14:35.

During the 57.5 °C open-door challenge, the door was opened at 2:00 PM and closed at 2:07 PM, which gave an open-door period of about 7 minutes. All sensors subsequently returned to the 55–60 °C operating band. The longest recovery was on the order of 25 minutes according to the executed study record, with the protocol identifying the last sensor to return to the acceptable range. Extended data logging after recovery documented re-establishment of the steady thermal field.

The Open Door Study with Loaded Chamber data at 57.5 °C is given in Figure 11.

LN	CH	Value	Maximum	Average	Minimum	Units	CH description
1	1		57.02	56.72	50.68	C	Sensor 1
2	2		58.58	57.88	52.47	C	Sensor 2
3	3		57.24	56.87	50.61	C	Sensor 3
4	4		56.69	56.27	49.97	C	Sensor 4
5	1		57.58	57.29	51.71	C	Sensor 5
6	2		59.51	58.22	53.99	C	Sensor 6
7	3		57.02	56.57	49.46	C	Sensor 7
8	4		56.49	55.97	47.91	C	Sensor 8
9	1		57.72	57.30	50.67	C	Sensor 9
10	2		57.57	56.96	42.67	C	Sensor 10
11	3		58.04	57.69	51.18	C	Sensor 11
12	4		57.40	56.94	48.01	C	Sensor 12
13	1		56.79	55.98	38.35	C	Sensor 13
14	2		56.97	56.36	47.77	C	Sensor 14
15	3		57.50	57.11	49.91	C	Sensor 15

Fig.11. Open Door Study with Loaded Chamber data at 57.5 °C

The somewhat longer recovery time compared with cooling mode, 17 minutes versus 14 minutes, may be attributable to the physics of heat transfer, including the lower density of warm air, or to controller settings that limit heating power to avoid hazardous overshoot at the final stage of recovery.

IV. DISCUSSION

4.1. Validity of the Temperature Bracketing Strategy

The results obtained strongly support the hypothesis that demonstrating control at boundary points provides sufficient evidence of system performance across the entire range. A system that successfully maintains uniformity within ±0.5 °C at 5 °C, where the resistance of dense air and the risk of icing are high, and ±0.8 °C at 57.5 °C, where heat losses through the cabinet are substantial, cannot physically be expected to perform worse at an intermediate temperature such as 37 °C. At mid-range temperatures, the temperature gradient ΔT between chamber and ambient is smaller, reducing stress on actuators and insulation. At the lower boundary of 5.0 °C, the chamber-to-ambient ΔT is approximately 15 °C (ambient at 20 °C), imposing maximum cooling demand on the compressor. At the upper boundary of 57.5 °C, ΔT reaches approximately 37.5 °C, imposing maximum heating demand and peak insulation load. At an intermediate setpoint such as 37 °C, ΔT is approximately 17 °C in cooling mode or effectively zero near ambient crossover - neither boundary condition is approached. This thermodynamic gradient argument, consistent with established heat transfer principles [16], provides the scientific foundation for boundary-point qualification.

In the present qualification program, this inference was not supplemented by separate empirical mapping at intermediate setpoints. The conclusion regarding intermediate temperatures was derived from the approved bracketing rationale, from the successful studies at the two thermal boundaries, and from the use of tighter acceptance bands at those boundary conditions than those assigned to routine intermediate setpoints in the qualification rationale.

This observation is consistent with ICH Q1D, which explicitly permits the use of bracketing to reduce the scope of stability studies [6]. Applying this approach to equipment qualification represents a logical extension of Quality Risk Management principles, enabling the reduction of validation workload without compromising quality.

Analysis of stability graphs also permits inferences about the type of cooling technology used and its suitability for various GxP applications. Nearly perfectly flat lines at 5 °C, absence of typical on/off compressor pulsation, suggest the use of a high-quality inverter compressor.

The results of the case study, recovery within 14–17 minutes, indicate that a modern system can provide acceptable recovery dynamics, challenging the cliché of the inherent weakness of thermoelectric systems.

4.2. The Role of Lozd and Aerodynamics

The study showed that the loaded chamber is a more stringent test than the empty chamber. From an engineering perspective, the introduction of a distributed chamber load changes both convective flow structure and transient heat exchange within the cabinet. Shelf loading creates partial obstruction of local air pathways and may reduce the rate of mixing between front and rear zones and between upper and lower regions. Added thermal mass also alters transient response by increasing the amount of energy required for the chamber to change temperature after control actions or door-opening disturbances. For this reason, a loaded chamber study has direct value for evaluation of spatial gradients, local stagnation effects, and recovery behavior under routine operating conditions.

The qualification protocol incorporated this engineering concern into the performance test design by requiring a simulated full-load condition distributed across the shelves. The resulting data showed that the chamber retained control within the predefined acceptance limits despite the flow resistance and thermal buffering associated with the loaded configuration.

The expansion of the temperature range from 0.4 °C (empty) to 2.0 °C (loaded) at 5 °C underscores the importance of proper product placement. This finding supports the necessity of strict loading procedures. Validation should be performed using a worst-case loading scenario, maximally dense, airflow-blocking, to define the operational design space. Ignoring this aspect and validating only an empty chamber are common errors that lead to audit findings.

4.3. Interpretation of Recovery Data and MKT

Door-opening recovery graphs carry critical information about system health. Confidence in the resulting qualification data was supported by the control features of the study design. All mapping instruments were calibrated and traceable to NIST or equivalent standards. Post-use verification at three temperature points confirmed that logger drift remained within the predefined acceptance limit of ±0.5 °C. The studies were performed with parallel observation of the chamber monitoring point and the controller probe. Test execution, review, and approval were documented within the qualification package by validation and quality personnel. These controls support reliability of the reported thermal profiles and of the associated interpretation of chamber performance.

If during initial qualification recovery takes 14 minutes, and at requalification after one year it takes 20 minutes, this is an early signal of component degradation, refrigerant leakage, semiconductor wear, fouled radiators, even if steady-state temperature remains within specification.

Based on the qualification data reported here, a preliminary set of lifecycle recovery benchmarks is proposed for dual-use refrigerated incubators of comparable chamber volume:

(1) Within-spec: recovery within the initial qualification baseline ± 20%;

(2) Investigation trigger: recovery exceeding initial baseline by more than 20% but less than 50%, requiring HVAC check, door seal inspection, and condenser cleaning;

(3) Requalification trigger: recovery exceeding initial baseline by 50% or more, or failure to recover within 30 minutes. These thresholds are illustrative and should be adapted based on equipment-specific qualification data. Their adoption would embed proactive degradation detection into the requalification lifecycle.

Use of Mean Kinetic Temperature, the MKT, to assess such excursions is acceptable but subject to

limitations. As noted in [9], MKT cannot be used to justify systematic deviations or poor equipment performance. However, in brief door-opening tests such as this, MKT calculations indicate that the effective thermal load on the product changes minimally, allowing a justified conclusion of no impact on product quality. It should be noted that MKT interpretation is product-specific: the activation energy (Ea) applied in the Haynes equation must reflect the degradation kinetics of the stored material. For a dual-use incubator accommodating diverse product classes, a single Ea value cannot be universally applied. Validation specialists should confirm Ea applicability on a per-product basis, consistent with ICH Q1A guidance on stability study design [26].

4.4. Lifecycle Management

Validation should not be a one-off event. The Lifecycle-Based Qualification Strategy for Dual-Use Refrigerated Incubators is shown schematically in Figure 12.

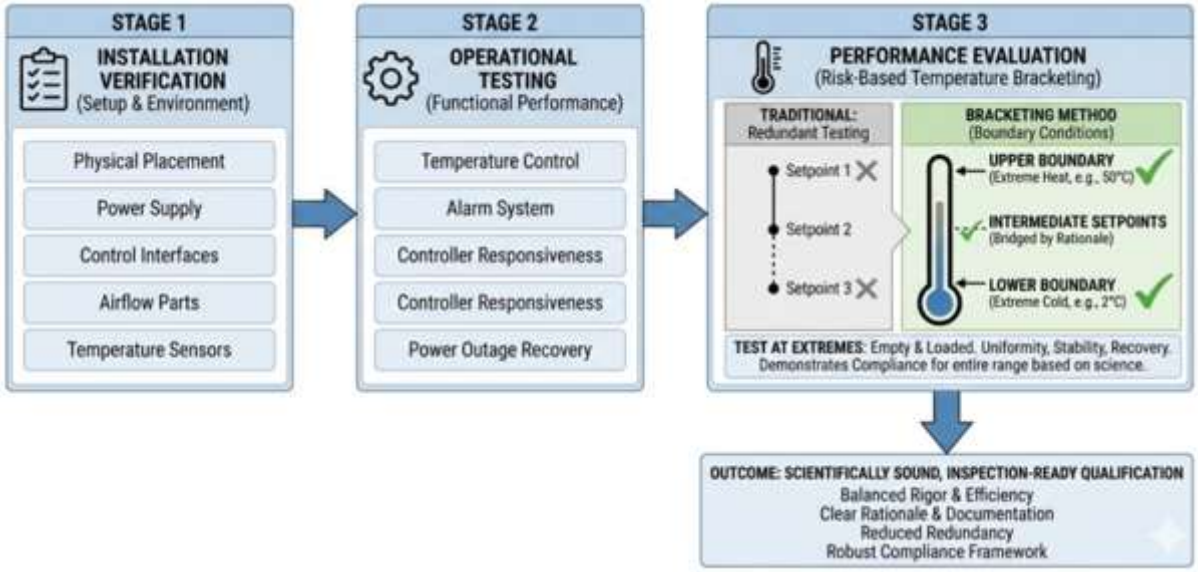

Fig.12. Lifecycle-Based Qualification Strategy for Dual-Use Refrigerated Incubators

The diagram illustrates a cyclical approach. Data obtained during the PQ phase, identification of hot and cold spots, should be used to select locations for permanent monitoring sensors. This implements the principle of risk-based monitoring placement [10]. Instead of placing the sensor at the geometric center, it should be located at the point with the greatest variability or the most extreme values during mapping.

4.5. Scientific Novelty and Comparison With Modern Literature

Recent studies on controlled thermal chambers show a clear move toward high-resolution monitoring, distributed sensing, and digital traceability, yet they mainly address observation of operating variables rather than transferability of qualification evidence across a full working range, as seen in incubator monitoring systems based on sensor networks and alert logging [11, 12]. Experimental work on multilevel incubator sensing and compact cell-culture mini-incubators confirms that spatial temperature heterogeneity remains a design-relevant issue even in small chambers, since local flow stagnation and wall-adjacent gradients persist despite acceptable average values [13, 14]. A transport incubator developed for spaceflight payloads further demonstrates that stable thermal control can be achieved under demanding boundary conditions, yet that work is centered on hardware robustness and power continuity rather than qualification logic for intermediate setpoints [15]. Against this background, the present study advances the field by linking chamber physics, boundary-point mapping, loaded-state behavior, and lifecycle verification into one qualification framework for a dual-use refrigerated incubator, which is a combination not established in the recent literature surveyed here.

The thermophysical rationale for boundary-point

qualification is supported by current heat-transfer literature, which shows that the largest chamber-to-ambient temperature differences amplify conductive and convective losses and intensify non-uniformity within refrigerated spaces [16]. Computational studies of cold rooms also show that storage geometry and stacking pattern reshape airflow pathways and create measurable local temperature deviations, even when the global mean temperature appears acceptable [17]. Work on incubator airflow optimization reaches the same physical conclusion, since air inlet geometry, flow redistribution, and internal mixing directly determine the temperature difference across the occupied volume [18]. The novelty of the present study lies in converting these engineering principles into a qualification argument, where the lowest and highest operating setpoints are treated as physically justified worst cases for cooling stress, heating stress, insulation load, stratification, and control-loop transition.

Recent literature also indicates that dynamic disturbances deserve independent analytical weight, since real thermal performance cannot be inferred from steady-state mapping alone. A controlled Peltier-based climate system reproduced time-varying cold-chain profiles with improved transient tracking through advanced control architecture, which underscores the scientific value of studying recovery kinetics rather than static compliance alone [19]. CFD-supported analysis of pharmaceutical storage after ventilation failure showed that recovery of design temperature depends on strongly non-uniform flow structures and inlet mass flow conditions, which means that restoration time is a physically meaningful indicator of system health [20]. Reviews of ultra-low-temperature freezers likewise identify door-opening recovery time as a critical performance metric because it determines how fast the unit returns to the specified temperature domain after disturbance [21]. The present work extends this line of research by proposing recovery rate as a qualification-relevant attribute for dual-use incubators, with direct interpretation in terms of degradation detection, risk to stored materials, and future requalification strategy.

A further gap in the literature concerns the translation of airflow and temperature models into operational verification rules for small dual-regime chambers. Studies on forced-air cooling of tomatoes and commercial storage of Chinese cabbage show that packaging, venting, and internal heat generation alter local cooling efficiency and temperature distribution in ways that cannot be captured by a single-point reading [22, 23]. A hybrid model combining computational fluid dynamics and machine learning demonstrates that full-field thermal prediction can support management of cold-storage boxes under changing process conditions, including applications relevant to frozen food and vaccines [24]. Small cold-storage systems with improved insulation architecture also confirm that chamber uniformity is a design variable that must be evaluated in relation to internal heat-transfer paths and load distribution rather than nominal setpoint alone [25]. The specific contribution of the present study is therefore not limited to showing that the tested incubator passed qualification. It establishes a literature-grounded method for extrapolating qualification evidence from boundary setpoints to intermediate conditions through combined analysis of steady-state uniformity, loaded-chamber aerodynamics, and door-opening recovery within a lifecycle verification strategy.

V. CONCLUSIONS

This study demonstrates that temperature bracketing at +5.0 °C and +57.5 °C, combined with loaded-chamber and door-recovery testing, provides a scientifically sufficient and regulatorily defensible basis for qualifying the full operating range of a dual-use refrigerated incubator. Efficiency gains are achieved not by reducing the evidentiary base, but by rationalizing it: testing effort is concentrated at thermal extremes that represent worst-case conditions for cooling stress, heating load, stratification, and control-loop transition. The proposed framework harmonizes ICH Q9 risk management with lean qualification logic, and establishes recovery rate as a lifecycle performance indicator capable of detecting equipment degradation before steady-state metrics are affected.

Empirical mapping data at boundary setpoints of +5.0 °C and +57.5 °C using 15 independent calibrated loggers delineate a consistent performance envelope: in the empty chamber at +5 °C, the system exhibits a narrow range of readings and pronounced spatial

uniformity without indications of hazardous dips below the critical freezing threshold, whereas under load the range predictably broadens due to aerodynamic resistance and vertical gradient formation, yet temperature remains confidently within the 2–8 °C specification with a substantial margin. In heating mode at +57.5 °C, comparably robust stability is observed, with no pronounced peripheral cold zones even at large ΔT relative to ambient, which is indirectly consistent with adequate insulation and PID tuning that prevents overshoot. Collectively, this is not merely a set of satisfied acceptance criteria but a reconstruction of how the system distributes risk across volume and time under various thermal loads.

Dynamic open-door/recovery tests clarify that quality for dual-use equipment is not exhausted by steady-state metrics: brief excursions shape the product's exposure profile, and recovery rate emerges as a critical parameter that can serve as an early indicator of degradation. In cooling mode under load, the short frontal temperature spike upon door opening is, as expected, more pronounced than in the chamber depth, after which the system returns to the specified band in less than a quarter of an hour; in heating mode, a mirror image is observed, with a temperature drop and somewhat longer recovery, which is consistent with heat-transfer physics and conservative controller logic limiting overshoot risk in the final recovery phase. At the same time, the boundaries of acceptable MKT use are delineated: it is appropriate as a tool for interpreting brief episodes in product impact assessment, but cannot serve as an indulgence for systematically poor equipment performance.

Ultimately, the study articulates a practice-oriented yet scientifically verifiable position: temperature bracketing for small dual-use laboratory systems is valid provided that boundary setpoints are rigorously selected as worst cases, tests are conducted in three modes, empty chamber, loaded chamber, and recovery, and results are linked to a risk-based monitoring lifecycle. The key conclusion is that demonstration of fitness for use shifts toward demonstrating robustness at the boundaries and in dynamics, while efficiency is achieved not by shrinking the evidentiary base but by rationalizing it: PQ data are used for risk-based placement of RMS points in zones of maximal variability rather than in an aesthetically appealing geometric center. In this way, the proposed approach harmonizes GxP expectations with lean logic, minimizing regulatory vulnerability up to the risk of FDA 483-level findings in cases of weak program justification while simultaneously reinforcing process understanding as the central criterion of contemporary qualification.

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