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BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2026.3004
Braz. J. Radiat. Sci., Rio de Janeiro
2026, 14(1) | 01-17 | e3004
Editor: Prof. Dr. Bernardo Maranhão Dantas
Editor: Prof. Dr. Alfredo Lopes Ferreira Filho
Submitted: 2025-09-04
Accepted: 2025-11-19

Original Article

Practical leak detection and leak rate measurement applied to a hot cell for radiopharmaceuticals preparations in a GMP compliant Laboratory

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Abstract: Hot cells are containment systems used in radiopharmaceutical production, designed to ensure radiation protection and product quality. Leak tightness testing is a mandatory requirement under CNEN NN 6.13 standard, as it directly impacts worker safety, environmental protection, and product integrity. This study aimed to perform a practical pressure change leak test in a hot cell used for radiopharmaceutical preparations, in accordance with applicable technical standards. The pressure change method was applied using pressures between 1.6 and 2.5 higher the working pressure for a testing time of 15 minutes. The method proved practical, requiring no highly specialized equipment, although its sensitivity to temperature highlights the importance of high-precision thermometer. Results showed that while the method is suitable for ISO 10648-2:1994 Class 2 compliance, it may not be adequate for Class 1 due to the risk of mathematically inaccurate results under temperature variations. Multiple rounds of leak detection and repair were required to achieve the required containment level. Overall, the DC, RIC, CIC, and Solid Waste chambers met Class 2 leak tightness requirements, being suitable for use in radiopharmacy facilities.

Keywords: hot cell; leak tightness; pressure change method; radiopharmaceutical;

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Detecção de vazamentos e medição da taxa de vazamento em célula quente para produção de radiofármacos em um laboratório BPF

Resumo: Células quentes são sistemas de contenção utilizados na produção de radiofármacos, projetadas para garantir a proteção radiológica e a qualidade do produto. A realização de testes de estanqueidade é um requisito obrigatório segundo a norma CNEN NN 6.13, pois impacta diretamente a segurança dos trabalhadores, na proteção ambiental e na integridade do produto. Este estudo teve como objetivo realizar um teste prático de estanqueidade em uma hot cell utilizada para a produção de radiofármacos, de acordo com as normas técnicas aplicáveis. O método da mudança de pressão foi aplicado, utilizando pressões entre 1,6 e 2,5 vezes a pressão de trabalho por 15 minutos. O método demonstrou ser prático, não exigindo equipamentos altamente especializados, embora sua sensibilidade à temperatura recomende o uso de termômetros de alta precisão. Os resultados mostraram que, embora o método seja adequado para conformidade com Classe 2, ele pode não ser apropriado para Classe 1 devido ao risco de resultados matematicamente imprecisos provenientes principalmente de variações de temperatura. Foram necessárias múltiplas rodadas de detecção e reparo de vazamentos para alcançar o nível de contenção exigido. No geral, as câmaras DC, RIC, CIC e Solid Waste atenderam aos requisitos de estanqueidade da Classe 2, estando adequadas para utilização em instalações de radiofarmácia.

Palavras-chave: hot cell; teste de estanqueidade; método de mudança de pressão; radiofármacos;

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1. INTRODUCTION

Nuclear medicine is a medical field that uses radioactive isotopes to diagnose and treat various diseases [1]. Radioactive isotopes are combined with organic or biological molecules to form radiopharmaceuticals, allowing diseased tissues and specific biological sites in the human body to be directly exposed to radioactivity. [2] [3].

Radioisotopes and radiopharmaceuticals have been produced in Brazil by IPEN, the pioneering institution in this field, since 1963 [4]. Since then, the institution has been engaged in the development and production of various radiopharmaceuticals, contributing significantly to the progress of nuclear medicine. Examples include iodine-131, molybdenum-technetium (99Mo-99mTc), thallium-201 chloride, gallium-67 citrate, and iodine-131- and iodine-123-labeled metaiodobenzylguanidine (MIBG) [5]. To ensure quality, safety, and efficacy throughout all stages of radiopharmaceutical production, compliance with the Good Manufacturing Practices (GMP) standards [6] [7] [8] and the radiation safety and protection requirements of the Brazilian Nuclear Energy Commission (CNEN) are required [9] [10].

Hot cells are particular features equipment's used in the production of radiopharmaceuticals, allowing operations with different radiation emitters (alpha, beta, and gamma). The lead-shielded walls are designed to minimize operator exposure to radiation [11] [12]. The ventilation system includes particle filters to purify the air around the product, along with chemical filters to capture radioactive gas emissions[11] [13]. Operations are performed remotely using clamps or telemanipulators. Lead oxide glass windows attenuate ionizing radiation, allowing the operator to observe and intervene in the production process [14].

Hot cells are classified as containment enclosures, designed to maintain a specialized environment through an airtight barrier and prevent the release of products into the external environment **Erro! Fonte de referência não encontrada.**[15] [16]. Their interior

compartments typically operate under negative pressure to prevent the escape of hazardous substances, protecting workers, the public, and the environment, while maintaining radiological doses as low as reasonably achievable (ALARA) [13] [17].

Leak tightness is a fundamental measure of the quality of the containment enclosure barrier [18]. Leak testing of hot cells in radiopharmacy facilities is a mandatory requirement under CNEN NN 6.13 [19] standard.

Different methods can be employed to measure the leak rate of a containment enclosure according to ISO 10648-2:1994 [15] and ISO 14644-7:2004 [20], acceptance testing is specified at −1000 Pa for one hour. For pharmaceutical isolators, this pressure level may be excessive and could cause irreversible damage to rigid-wall designs. In practice, pressures of 1.5 to 5 times the normal operating level, applied for shorter durations, are generally sufficient for leak rate measurement [18] [21] [22] [23]. Short test reduces the impact of atmospheric pressure variations, since the rate of atmospheric pressure change is approximately 50 Pa/h. [22] [23].

This study aims to perform a practical pressure change leak test in a hot cell designed for radiopharmaceutical preparations, as part of the acceptance test, in accordance with applicable technical standards.

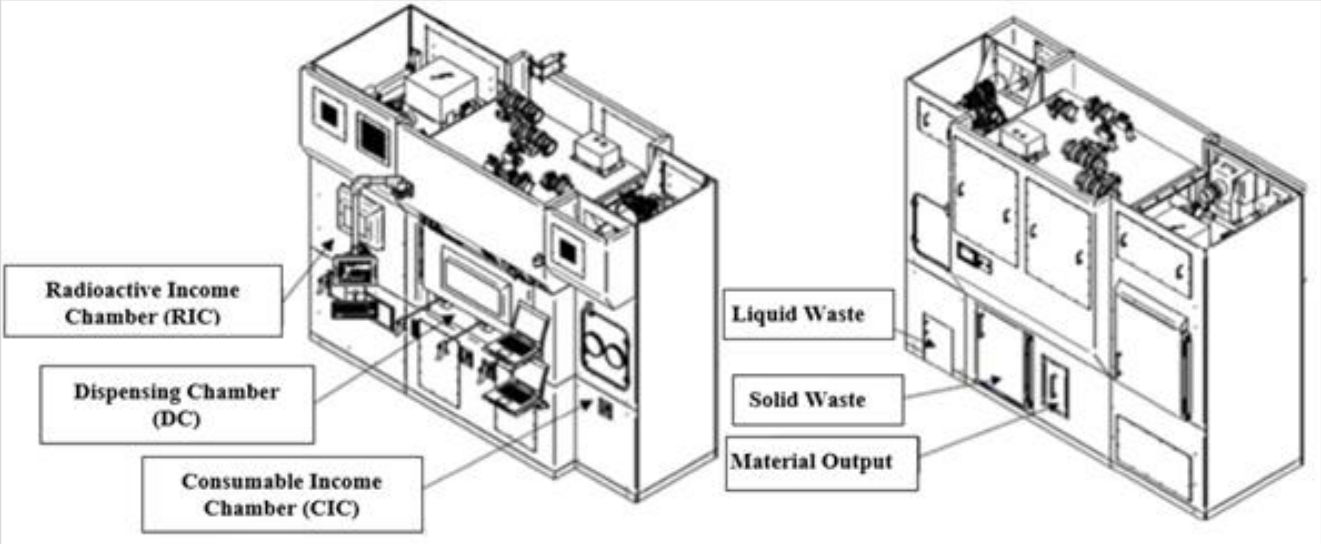
2. MATERIALS AND METHODS

The methodology was based on ISO 10648-2:1994 - Containment enclosures - Part 2: Classification according to leak tightness and associated checking methods; ISO 14644-7:2007 - Cleanrooms and associated controlled environments - Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments), to meet the requirements of CNEN NN 6.13 Safety and Radiological Protection Requirements in Centralized and Industrial Radiopharmacy Installations.

2.1. Equipment setup

The Talia model hot cell, manufactured by Comecer, is designed for filling, fractionation, sealing and calibration of radiopharmaceuticals in aseptic environment, in accordance with ISO 14644-1 [24] air cleanliness standards. The equipment consists of three internal chambers: Dispensing Chamber (DC), Radioactive Income Chamber (RIC), and Consumable Income Chamber (CIC), and three secondary chambers: Liquid Waste, Solid Waste, and Material Output (Figure 1).

Figure 1: Hot cell Talia front and back view.



Source: IPEN technical files.

The Talia hot cell was designed to provide grade A classification with unidirectional airflow in the Dispensing Chamber (DC), under a depression of -100 Pa. The chambers RIC, CIC Solid Waste and Material Output operates in grade B, under a depression of -150 Pa. The Liquid Waste chamber consists of a sealed reservoir and does not requires testing.

The internal chambers are manufactured from 3 mm thick AISI 304 stainless steel sheets. The glove ports and visors are manufactured from 20 mm thick acrylic sheets, and their sealing is achieved by a pneumatically actuated silicone gasket (Figure 2).

Figure 2: DC and RIC internal chambers with pneumatic gaskets.

Source: Author.

Their internal chambers have interfaces for utilities such as electrical outlets, compressed air, and nitrogen. Internally, they have electro pneumatic actuators for opening and closing doors and equipment such as a dose calibrator.

The Talia hot cell is installed in a GMP grade C cleanroom, with a grade D boundary to the external environment, completing the cleanliness cascade (Figure 3).

Figure 3: Clean area classification laboratory and Talia hot cell.

Source: IPEN technical files.

2.2. Leak detection

Larger leaks can be visually detected by applying a soap solution while the containment is pressurized with compressed air or inert gas. Visual smoke tests can also help identify leak

points. Smaller leaks can be detected using a tracer gas and a "sniffer" device. Gases such as helium or refrigerants are introduced into the containment to identify leakage points.

Joints, gaskets, valves, and connection points with the external environment, such as pneumatic and electrical interfaces, present a high probability of leakage and must be inspected.

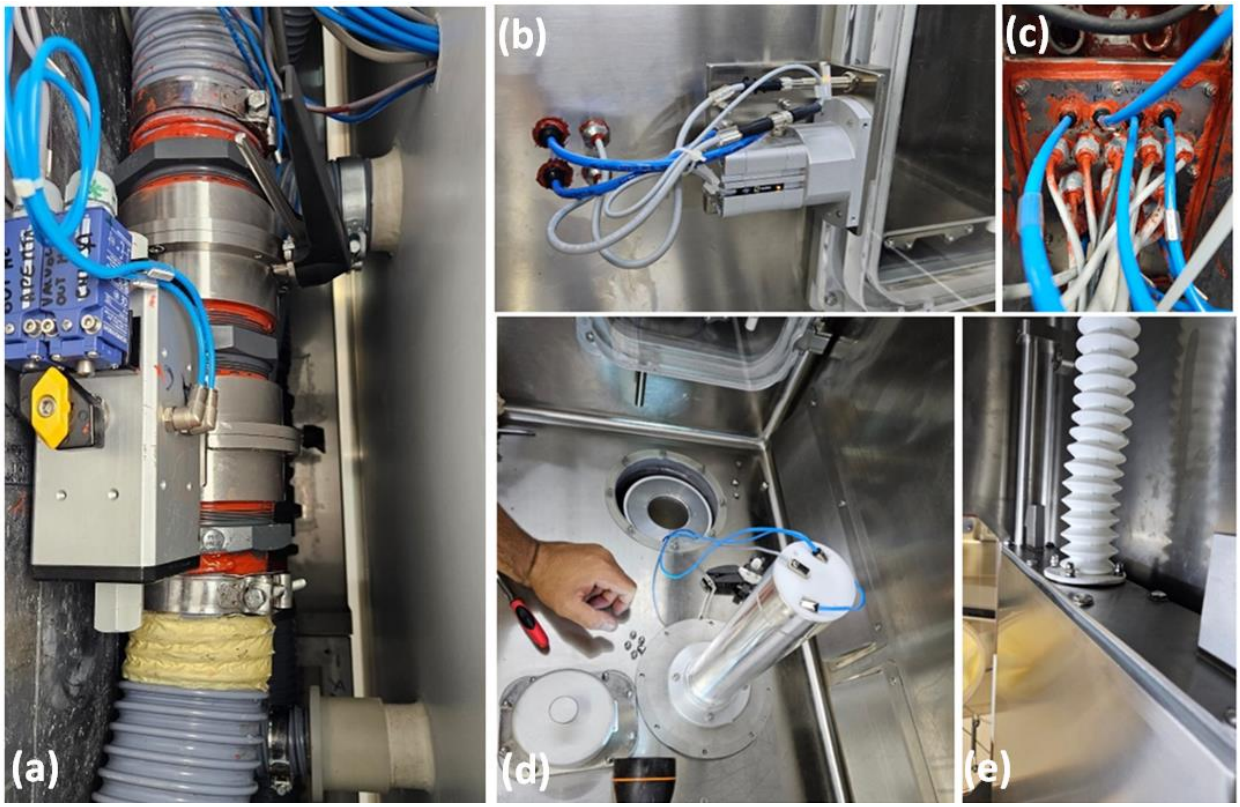
Leak detection was carried out on a macro scale, using smoke and soap solution tests. To identify small leaks, the chambers was filled with R22 refrigerant, pressurized with compressed air, and an electronic refrigerant detector was employed (Figure 4).

Figure 4: Leak detection with refrigerant R22.

Source: Author.

Leaks were identified and repaired in manual and pneumatic valves, hoses, electrical connections, and the dose calibrator. Pneumatic and electrical couplings were sealed with adhesive. A corrective inspection was performed on all interfaces connecting the hot cell to the external environment (Figure 5).

Figure 5: Leak repair in (a) vetilation valve, (b) eletropneumatic cylinder, (c) connectors, (d) dose calibrator and (e) covering joints.



Source: Author.

Multiple rounds of leak detection and repair were required to achieve the containment level.

2.3. Leak rate measurement

Leak tightness classes can be analyzed as a rate of volume loss per hour: Class 1: 0.05% volume loss/hour; Class 2: 0.25% volume loss/hour; Class 3: 1.0% volume loss/hour; Class 4: 10% volume loss/hour [18]. Table 1 shows the classification of containment enclosures, their applications and test methods indicated for each class.

Table 1: Classification of containment enclosures according to their hourly leak rate.

Class	Hourly leak rate T_f [h^{-1}]	Application example	Test methods
1	$\leq 5x10^{-4}$	Containment enclosure with controlled atmosphere under inert gas conditions	Oxygen, pressure change[1] or Parjo
2	$< 2{,}5x10^{-3}$	Containment enclosure with controlled atmosphere under inert gas conditions or with permanently hazardous atmosphere	Oxygen, pressure change or Parjo
3	$<10^{-2}$	Containment enclosure with permanently hazardous atmosphere	Oxygen, pressure change or constant pressure
4	$< 10^{-1}$	Containment enclosure with atmosphere which could be hazardous	Constant pressure

[1]: The pressure change method is recommended for Class 1 by ISO 14644-7:2007, but it is not recommended by ISO 10648-2:1994. Source: ISO 10648-2:1994 and ISO 14644-7:2007

In accordance with CNEN NN 6.13, radiopharmaceutical hot cells must comply with a minimum containment performance of Class 4. However, the equipment manufacturer specifies Class 2 as the design requirement. To meet the manufacturer's Class 2 requirement, the pressure change method will be used.

The hourly leak rate is calculated using the Equation 1:

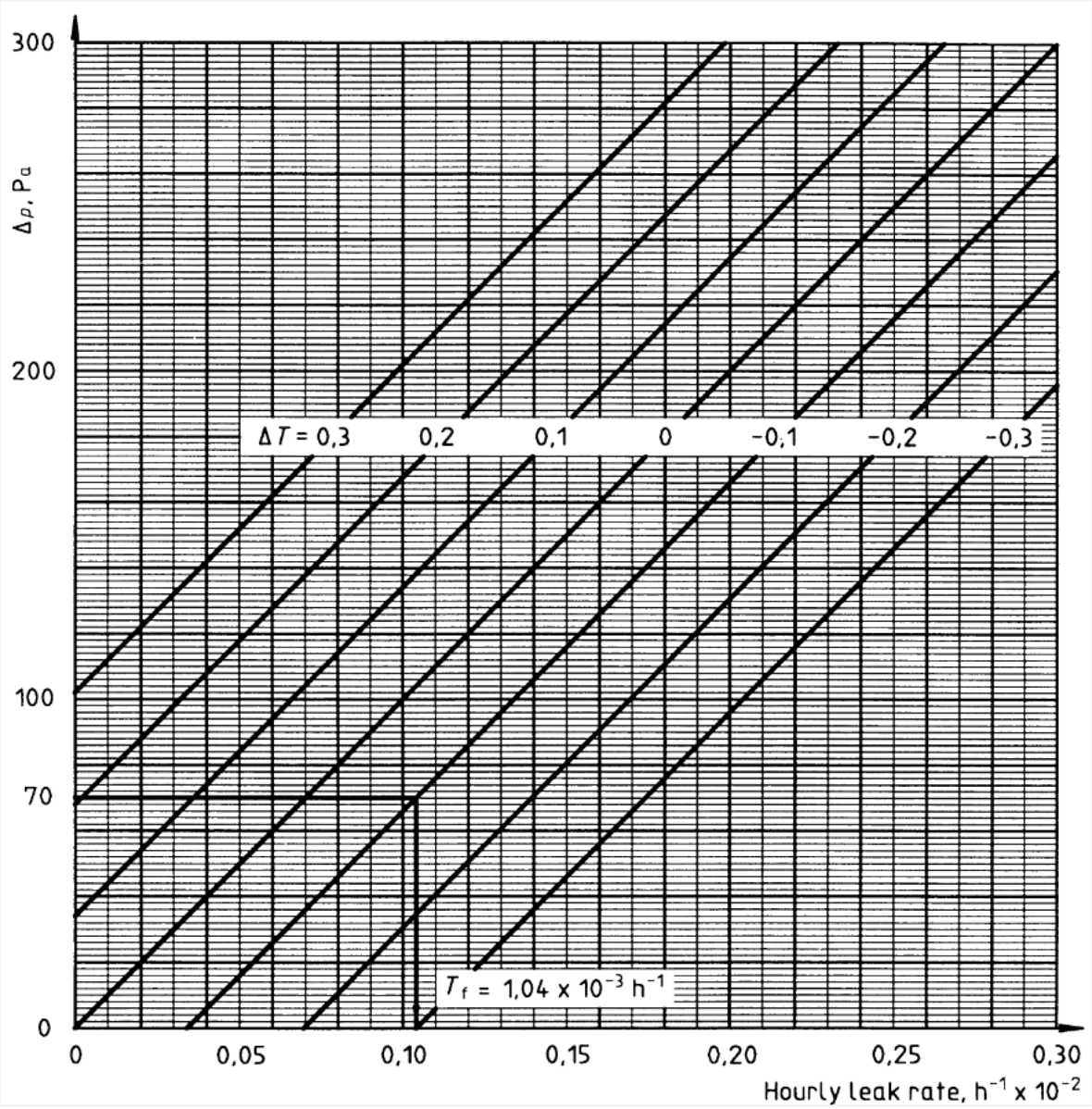
$$T_f = \frac{60}{t} x \left(\frac{P_n T_0}{P_0 T_n} - 1\right) \quad (1)$$

Where:

T_f - hourly leak rate;
t - test duration, in minutes;
$T_{0/n}$ - temperature at beginning/end of test, in Kelvin;
$P_{0/n}$ - absolute pressure (ambient pressure minus underpressure) at beginning/end of test, in Pascal

Equation 1 can be interpreted graphically (Figure 6) and can also be used to determine T_f, where Δp on the Y axis is the variation in containment absolute pressure ($P_n - P_0$) and ΔT lines is the variation in temperature ($T_n - T_0$). The following figure illustrates the determination of T_f for Δp = 70 Pa for ΔT = -0.1 °C.

Figure 6: Nomogram for graphical determination of Tf



Source: ISO 10648-2:1994

Changes in atmospheric pressure and room temperature can affect the quality of the results. During the test, laboratory doors should not be opened or closed, the internal equipment lights should be off in advance to prevent heating, the pressurization and HVAC systems should be checked. Moments of significant temperature fluctuations along the day should be avoided. Gloves and clamp covers should be removed and glove ports must be sealed for testing, to avoid undesirable volumetric variations. Before starting the leak test, the temperature in the containment and in the room must be allowed to stabilize.

To perform the negative pressure test, the containment air inlet valve must be closed to ensure that the exhaust system generates the required negative pressure; if necessary, an auxiliary vacuum device should be used. Subsequently, the pressure should be adjusted below the target level, and then the outlet valve should be closed, allowing the internal pressure to stabilize.

The pressure and temperature of the containment shall be continuously monitored throughout the test through the equipment's pneumatic pressure monitoring point. The thermometer must be positioned inside the chamber.

For the acceptance test, a negative pressure of −250 Pa will be applied. Following stabilization, the pressure rise will be monitored over a 15-minute period.

Atmospheric pressure will be considered constant at 101,000 Pa. The chamber's internal temperature must not vary by more than ±0.3 °C.

2.4. Instruments

A Fluke 922 micro manometer was used to measure differential pressure. A Traceable 4239 thermometer was used to measure temperature. Both were calibrated.

To measure the negative pressure inside each chamber, the pneumatic connector available on the hot cell's technical panel was used. To measure the temperature, the thermometer was positioned in the center of each chamber.

The use of a high-precision thermometer increases the accuracy of the test, considering that a variation of 0.1 °C in the internal temperature corresponds to 35 Pa, being compensated in Equation 1 (Figure 6).

3. RESULTS AND DISCUSSIONS

Ensuring the integrity of containment enclosures is of fundamental importance, as it directly impacts the safety of operators, the reliability of radiopharmaceutical production, and compliance with regulatory requirements [7] [8]. The leak rate measurement test posed a significant challenge to meeting the acceptance criteria, as initial results were below the limit for all chambers, requiring several rounds of leak detection and repair.

All tested compartments (DC, RIC, CIC, Solid Waste, and Material Output) were approved in Class 2 tightness, as the hourly leakage rate (T_f) in all cases remained below the acceptance criterion ($<2.5\text{x}10^{-3}$ h^{-1}). The test results after correction of the leakage points are presented in Table 2.

Table 2: tightness test results.

Chamber	Chamber pressure [Pa]		Δp [Pa]	Chamber temperature [°K]		ΔT [K]	Hourly leak rate T_f [h^{-1}]	Acceptance criteria [h^{-1}]	Result
	P_0	P_{15}		T_0	T_{15}				
DC	-250	-203	47	294.52	294.54	0.02	$1{,}59\text{x}10^{-3}$	$< 2{,}5\text{x}10^{-3}$	Approved
RIC	-250	-247	3	294.75	294.97	0.22	$-2{,}86\text{x}10^{-3}$	$< 2{,}5\text{x}10^{-3}$	Approved
CIC	-250	-225	25	292.15	292.11	-0.04	$1{,}54\text{x}10^{-3}$	$< 2{,}5\text{x}10^{-3}$	Approved
Solid Waste	-250	-248	2	290.37	290.35	-0.02	$3{,}55\text{x}10^{-4}$	$< 2{,}5\text{x}10^{-3}$	Approved
Material Output	-250	-201	49	290.08	290.08	0.00	$1{,}95\text{x}10^{-3}$	$< 2{,}5\text{x}10^{-3}$	Approved

Source: Author.

The measured T_f values ranged from $-2.86\text{x}10^{-3}$ h^{-1} (negative) to $1.95\text{x}10^{-3}$ h^{-1}, all within the established limit. The Solid Waste compartment presented the lowest leakage rate ($3.55\text{x}10^{-4}$ h^{-1}), indicating better sealing performance among the tested chambers. The negative rate observed in the RIC occurs due to a slight pressure gain (from -250 to -247 Pa) resulting from internal temperature variation, which can be interpreted as a measurement artifact, but still within acceptance criterion. The conditions for this analysis will be addressed later in this topic.

The largest temperature variation was observed in RIC compartment (0.22 °C). All temperature variations across the chambers between time 0 (T_0) and 15 minutes (T_{15}) are

within the standard range (0.3 °C), indicating stable environmental conditions during testing. Such stability is relevant, since temperature fluctuations can affect pressure and consequently the hourly leakage rates.

The test was consistently started at -250 Pa (P_0) for all compartments, and the final pressure (P_{15}) was not higher than the working pressure (-100 Pa for DC and -150 Pa for the others), in accordance with the recommendation of Coles (2012) [18], confirming that the test method was standardized.

The duration of 15 minutes was sufficient to maintain the chamber's internal temperature within the variation limit of ± 0.3 °C and also minimized possible variations in atmospheric pressure, which was considered constant during the test, according to Coles (2012) [18], Coles and Neiger (2004) [21] and Weijing et al. (2019) [22].

The RIC chamber has few interfaces with the external environment, minimizing the chance of leaks, and presented the lowest pressure increase. The mathematical sign inversion observed in the RIC chamber leak rate is attributed to the increase in chamber temperature combined with the low-pressure fluctuation during the test (the increase of 0.22 °C in the internal temperature of the RIC chamber corresponds to a pressure increase of 77 Pa).

The graphical method represented in Figure 6 illustrates the mathematical limitation of Equation 1 when applied to combined values of low Δp (3 Pa) and high ΔT (0.22 °C), as is the case of the RIC chamber. Figure 6 shows that the minimum possible value for the ΔT = 0.2 °C line is approximately Δp = 70 Pa (or 77 Pa for the 0.22 °C line). In other words, for an hourly leak rate of zero, a temperature variation of 0.22 °C would correspond to an increase of 77 Pa in the internal pressure.

For the RIC chamber, the thermal correction factor is equivalent to a pressure variation of 77 Pa, which is much greater than the measured pressure drop of 3 Pa, producing a negative signal in the mathematical calculation. This does not mean that the chamber is gaining gas, but rather that the method has gone beyond its range of validity. This

mathematical limitation justifies its recommended use by ISO 14644-7:2004 for Class 1 leak tightness measurements (in certain cases), while ISO 10648-2:1994 does not recommend its application. However, the result may still be considered valid for demonstrating compliance with Class 2 standards, as is the subject of this study.

The Solid Waste chamber is small and has few interfaces with the external environment, which contributes to obtaining an hourly leak rate consistent with Class 1 ($\leq 5x10^{-4}$ h^{-1}). Even with a small internal pressure fluctuation ($\Delta p = 2$ Pa) due to the stability of its internal temperature (ΔT = -0.02 °C), its T_f value can still be determined using the graphical method, serving as a suitable example of the application of this method for Class 1 leak tightness.

4. CONCLUSIONS

The tests quantitatively demonstrated that the DC, RIC, CIC, and Solid Waste chambers complied with the regulatory requirements for Class 2 leak tightness in the acceptance test.

The pressure change method is practical and does not require highly specialized equipment; however, due to the method's high sensitivity to temperature, the use of a high-precision thermometer increases the accuracy of the test.

The use of pressures between 1.6 and 2.5 times the working pressure for a period of 15 minutes was sufficient to perform the measurements without compromising the physical integrity of the equipment.

The pressure variation method is satisfactory for Class 2 leak tightness measurements, but may present mathematical limitations when applied to Class 1 measurements due to temperature variation.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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