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GMP compliant microbiological monitoring in hot cells for radiopharmaceuticals preparations

Monitoramento microbiológico em células quente para produção de radiofármacos em ambiente BPF

Monitorización microbiológica en celdas calientes para la producción de radiofármacos en entorno BPF

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ABSTRACT
Radiopharmaceuticals are produced inside specialized equipment named hot cell. The integration between cleanrooms and hot cells allows the radiopharmaceutical production to meet radiological protection and Good Manufacturing Practices (GMP) standards, as established by regulatory agencies. The installation of a hot cell in a pharmaceutical cleanroom is a complex activity that must be documented and validated through qualification protocols. This study aims to perform microbiological monitoring in a hot cell for aseptic radiopharmaceutical preparations at the IPEN radiopharmacy facilities, considering the active air, settle plates, contact plates, and indirect samples of gloves and clamps, as part of the equipment's Operation Qualification (OQ) protocol. Microbiological monitoring was performed in five chambers of the hot cell (grade A and B). Four of the five chambers presented microbiological contamination within the limits established by the standard for all tests performed. The fifth chamber (Material Outlet) presented technical and construction limitations that prevented the active air sample test from being performed, so the hot cell partially met the regulatory requirements. Complementary tests, such as air cleaning by concentration of non-viable particles, detection of leaks in filtration systems, and air flow testing will contribute to justify the classification of the Material Outlet chamber.

Keywords: hot cell, radiopharmaceutical, Good Manufacturing Practices (GMP), microbiological monitoring.

RESUMO
Radiofármacos são produzidos no interior de equipamentos especializados denominados células quentes. A integração entre salas limpas e células quentes permite que a produção de radiofármacos atenda aos requisitos de proteção radiológica e de Boas Práticas de Fabricação (BPF), conforme estabelecido por agências reguladoras. A instalação de uma célula quente em uma sala limpa farmacêutica é uma atividade complexa que deve ser documentada e validada através de protocolos de qualificação. Este estudo tem como objetivo realizar o monitoramento microbiológico de uma célula quente destinada a preparações radiofarmacêuticas assépticas nas instalações de radiofarmácia do IPEN, considerando os ensaios de ar ativo, placas de sedimentação, placas de contato e amostras indiretas de luvas e pinças, como parte do protocolo de Qualificação de Operação (QO). O monitoramento microbiológico foi realizado em cinco câmaras que compõem a célula quente (grau A e B). Quatro das cinco câmaras apresentaram contaminação microbiológica dentro dos limites estabelecidos para todos os testes realizados. A quinta câmara (Saída de Material) apresentou limitações técnicas e construtivas que impediram a realização do teste de amostra de ar ativo, de modo que a célula quente atendeu parcialmente aos requisitos microbiológicos. A realização de ensaios complementares como: limpeza do ar por concentração de partículas não viáveis, detecção de vazamentos em sistemas de filtragem, ensaios de fluxo de ar contribuirão para justificar a classificação da câmara de Saída de Material.

Palavras-chave: célula quente, radiofármacos, Boas Práticas de Fabricação (BPF), monitoramento microbiológico.

RESUMEN
Los radiofármacos se producen dentro de equipos especializados llamados celdas calientes. La integración de salas blancas y celdas calientes permite que la producción de radiofármacos cumpla con los requisitos de protección radiológica y Buenas Prácticas de Manufactura (BPM), según lo establecido por las agencias reguladoras. Instalar una celda caliente en una sala blanca

farmacéutica es una actividad compleja que debe documentarse y validarse mediante protocolos de calificación. Este estudio tiene como objetivo realizar el monitoreo microbiológico de una celda caliente destinada a preparaciones asépticas de radiofármacos en las instalaciones de radiofarmacia IPEN, considerando pruebas de aire activo, placas de sedimentación, placas de contacto y muestras indirectas de guantes y pinzas, como parte del protocolo de Calificación Operacional (CO). El monitoreo microbiológico se realizó en cinco cámaras que componen la celda caliente (grados A y B). Cuatro de las cinco cámaras presentaron contaminación microbiológica dentro de los límites establecidos para todas las pruebas realizadas. La quinta cámara (Salida de Material) presentó limitaciones técnicas y de construcción que impidieron las pruebas de muestreo de aire activo, lo que significa que la celda caliente cumplió parcialmente con los requisitos microbiológicos. Pruebas adicionales, como la limpieza del aire mediante concentración de partículas no viables, la detección de fugas en los sistemas de filtración y las pruebas de flujo de aire, ayudarán a justificar la clasificación de la cámara de Salida de Material.

Palabras clave: celda caliente, radiofármacos, Buenas Prácticas de Fabricación (BPF), monitoreo microbiológico.

1 INTRODUCTION

Nuclear medicine is a medical specialty that uses radioactive atoms for the diagnosis and treatment of several diseases [1]. Radioactive isotopes are linked to organic or biological molecules to create a radiopharmaceutical, which allows radioactivity to be directed to pathological tissues and specific biological targets in the human body [2] [3]. Radiopharmaceutical preparations must simultaneously comply with radiological protection requirements and microbiological principles [4] [5].

The IPEN was the pioneering institution in Brazil for the production of radioisotopes and radiopharmaceuticals, starting its production in 1963 [6]. Since then, the institution has developed and produced different types of radiopharmaceuticals, playing a fundamental role in the advancement of nuclear medicine.

Good Manufacturing Practices (GMP) are a mandatory and legal requirement that aims to ensure quality, safety, and efficacy during all stages of drug production [7] [8] [9]. For intravenous radiopharmaceuticals, specific guidelines must be met to ensure that they are sterile and free of bacterial endotoxins [2].

Radiopharmaceuticals are produced inside equipment named hot cells, which allow operations at different radiation levels (alpha, beta, and gamma) [10]. Their lead-shielded walls are designed to significantly reduce the radiation flow that reaches the operator [11]. The hot

cell's internal chambers operate constantly at negative pressure to contain the radioactive material [12]. The ventilation system has particle filters, which treat the air in contact with the product, and chemical filters that capture radioactive exhaust [10] [12].

High standards of environmental cleanliness and air quality necessary for aseptic radiopharmaceutical preparations are achieved by integrating the hot cell with a cleanroom, where pressure, temperature, and humidity maintain particulate (non-viable) and microbial (viable) contamination levels within specified limits [13].

Viable particle control does not ensure sterility of the product, as many microorganisms cannot be incubated on traditional agar. However, this methodology supports the release of production batches when based on continuous process monitoring [14] [15].

Hot cells and cleanrooms are designed to maintain aseptic conditions, but remain vulnerable to contamination from internal sources (such as tools, chemicals, operators, equipment, coatings, etc.) and external sources via the replacement air [16] [17]. The hot cell's ability to maintain contamination within established levels must be evaluated and validated through qualification protocols [18].

This work aimed to perform microbiological monitoring in a hot cell for aseptic radiopharmaceutical preparations, at the IPEN radiopharmacy facilities, considering the active air, settle plates, contact plates, and indirect samples of gloves and clamps, as part of the Operation Qualification (OQ) protocol.

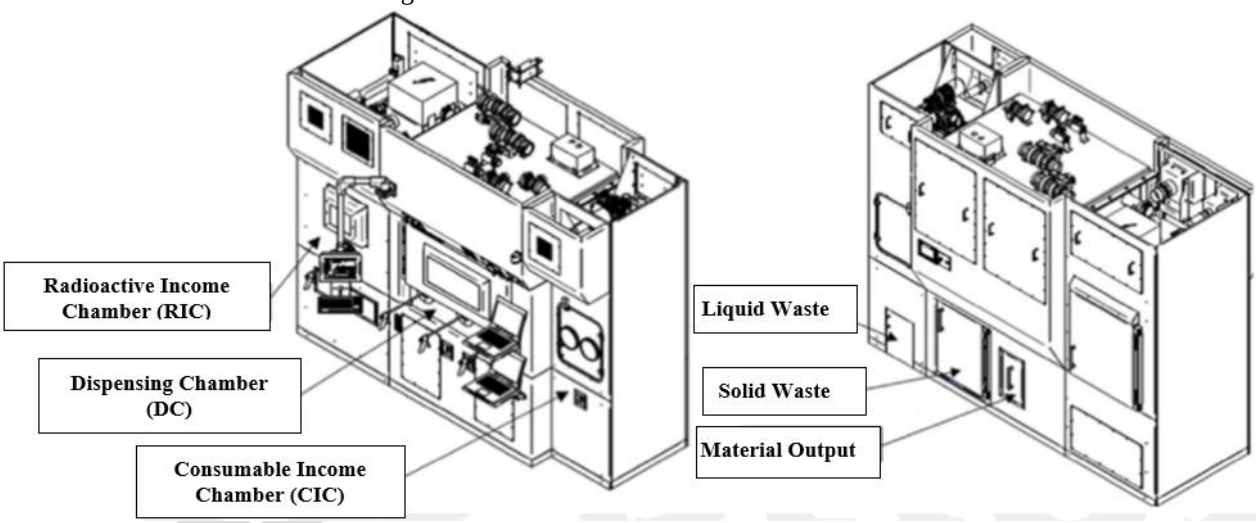
2 MATERIALS AND METHODS

The methodology was based on Good Manufacturing Practices (GMP) guidance for sterile drugs produced by aseptic processing established by the following regulatory agencies: the Brazilian Health Regulatory Agency (Anvisa) [19], the European Medicines Agency (EMA) [20], and the U.S. Food and Drug Administration (FDA) [21].

2.1 TALIA HOT CELL AND PHARMACEUTICAL CLEANROOM

The Talia model hot cell, manufactured by Comecer, was designed for filling, fractionation, sealing and calibration of radiopharmaceuticals in aseptic environment, according with ISO 14644-1 [22] air cleanliness standards. The equipment consists of three main 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, undated.

The Talia hot cell was designed to provide grade A classification under unidirectional airflow in the Dispensing Chamber (DC) and grade B in the remaining chambers. The Liquid Waste chamber consists of a sealed reservoir and does not requires classification.

The Talia hot cell is installed in a classified grade C cleanroom, with a grade D boundary to the external environment, completing the cleanliness cascade. (Figure 2).

Figure 2. Clean area classification laboratory and Talia hot cell.

Source: IPEN technical files, undated.

2.2 MICROBIOLOGICAL REQUIREMENTS

This study adopted the microbiological contamination limits established by ANVISA Normative Instruction 35/2019 on GMP for Sterile Drugs, along with their equivalent guidelines: Annex 1 – Manufacture of Sterile Medicinal Products by EMA and Sterile Drug Products Produced by Aseptic Processing by FDA. The contamination limits are presented in Table 1, expressed in Colony-Forming Units (CFU), for each of the four tests proposed by the standards.

Table 1. Maximum permitted microbial contamination levels.

Grade	Active air sample (CFU/m³)	Settle plates (Ø 90 mm - CFU/4 hours)	Contact plates (Ø 55 mm - CFU/plate)	Gloves/clamp (CFU/unit)
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Source: ANVISA, 2019 [19]

To sanitize the cleanroom, a 2.5% neutral detergent solution (Extran) was applied, followed by a 1:128 diluted Oxivir disinfectant solution. A sterile 70% isopropyl alcohol solution was applied to the internal surfaces of the hot cell. The cleaning procedures followed those established by the IPEN Radiopharmacy facilities, including gowning protocols to prevent personal contamination spread.

2.3 SAMPLING REQUIREMENTS

For active air sampling test, the MAS-100 NT device (Merck) was used with 90 mm Tryptic Soy Agar (TSA) culture plates. A total of 1000 liters of air (1 m³) was sampled at a flow rate of 100 liters per minute.

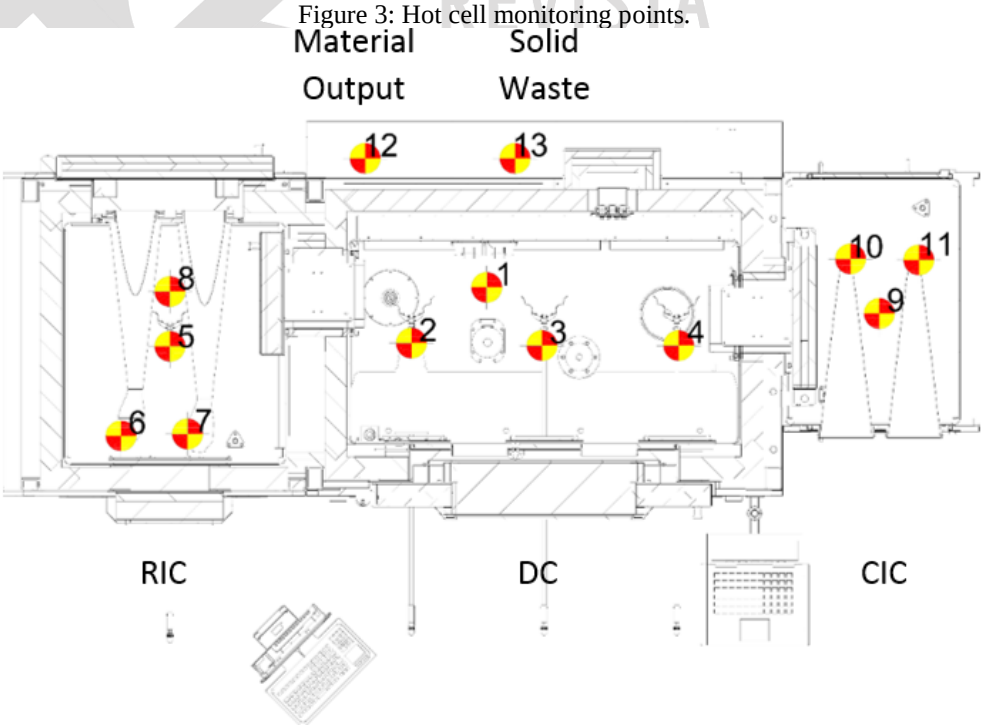
For the settle plate test, 90 mm TSA plates and Sabouraud dextrose agar (4%) plates were exposed for four hours.

For the contact plate test, 55 mm Rodac plates containing TSA were pressed against surfaces for 15 seconds.

Indirect sampling of gloves and clamps was performed using sterile swabs in peptone saline solution, which were transferred to 90 mm TSA agar culture plates.

The sampling points for microbiological monitoring were distributed at critical process points, across each hot cell chamber (Figure 3), for all four tests prescribed.

Figure 3: Hot cell monitoring points.



Source: IPEN technical files, undated.

After sampling, the plates containing Sabouraud agar were incubated at 22.5 ± 2.5°C for 7 days, while the plates containing TSA were incubated at 32.5 ± 2.5°C for 5 days.

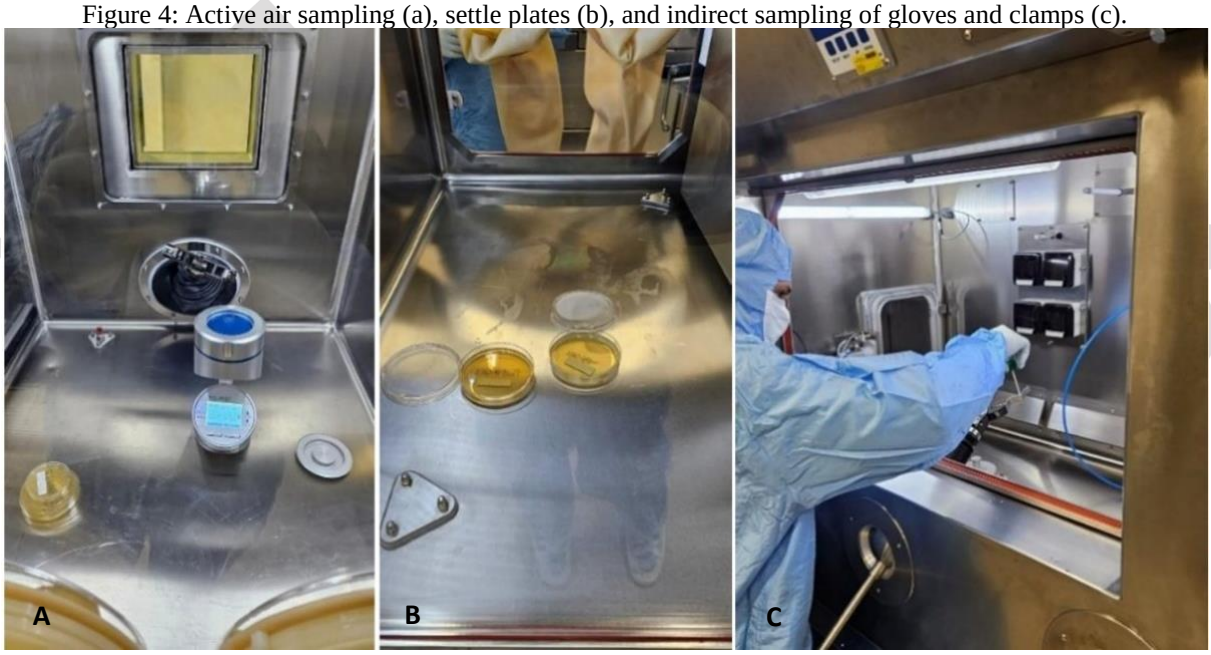
Materials were handled by trained and gowned operators. The plates were labeled and

incubated to prevent contamination transfer. The hot cell ventilation system remained active, ensuring air pressure was maintained between areas.

3 RESULTS AND DISCUSSIONS

The settle plate, contact plate, and indirect sampling tests for clamps and gloves were performed at all designated sampling points. The active air sampling test could not be performed at Point 12 - Material Output Chamber, the chamber's reduced dimensions prevented the accommodation of the MAS-100 NT instrument inside the chamber. The tests are illustrated in Figure 4.

Figure 4: Active air sampling (a), settle plates (b), and indirect sampling of gloves and clamps (c).



Source: Author.

After the incubation period, a visual inspection and CFU counting were performed. The results are presented in Table 2.

Table 2: Microbiological monitoring results.

Pt.	Grade	Position	Active air sample (CFU/m³)		Settle plates (CFU/4 hours)		Contact plates (CFU/plate)		Gloves/clamp (CFU/glove)		Conclusion
			Accept. criteria	Result	Accept. criteria	Result	Accept. criteria	Result	Accept. criteria	Result	
1	A	DC Chamber	<1	0	<1	0	<1	0	-	-	Approved
2	A	DC Left clamp	-	-	-	-	-	-	<1	0	Approved
3	A	DC Central clamp	-	-	-	-	-	-	<1	0	Approved
4	A	DC Right clamp	-	-	-	-	-	-	<1	0	Approved
5	B	RIC Chamber	10	0	5	0	5	0	-	-	Approved
6	B	RIC Left glove	-	-	-	-	-	-	5	1	Approved
7	B	RIC Right glove	-	-	-	-	-	-	5	4	Approved
8	B	RIC Central clamp	-	-	-	-	-	-	5	1	Approved
9	B	CIC Chamber	10	0	5	0	5	0	-	-	Approved
10	B	CIC Left glove	-	-	-	-	-	-	5	2	Approved
11	B	CIC Right glove	-	-	-	-	-	-	5	0	Approved
12	B	Material Output	10	Not performed	5	0	5	0	-	-	Inconclusive
13	B	Solid Waste	10	0	5	0	5	0	-	-	Approved

Legend: ‘’-‘’ test not compatible/proposed at this point.
Source: Author.

Microbiological monitoring allows the investigation and treatment of potential contamination in pharmaceutical production [23] [24]. The aim of this study was to perform microbiological monitoring in a hot cell for aseptic radiopharmaceutical preparations. The clean area classification tests confirmed that microbiological contamination was within the established limits for the DC, RIC, CIC, and Solid Waste chambers. For the Material Output Chamber, the settle plate and contact plate tests showed satisfactory results, while the active air sampling test could not be conducted due to the chamber’s dimensional limitations.

All GMP standards referenced in this study describe tests and limits for microbiological contamination in clean areas. However, only the EMA guideline specifies that the non-performance of any test must be appropriately justified.

Given the equipment's structural restrictions, the classification of the Material Output Chamber can be justified based on the results from the complementary settle plate and contact plate tests, which showed no microbiological growth (exceeding the grade B requirement). Additionally, considering the process flow inside the hot cell, the vial containing the finished radiopharmaceutical is sealed inside the DC and then transferred to the Material Outlet Chamber, eliminating the risk of product contamination and supporting the classification for this chamber.

The results suggest that the Material Output Chamber partially meets the regulatory requirements for a grade B classification. Due to limitations of the study, successfully passing tests such as air cleanliness classification by non-viable particle concentration, leak detection in installed filtration systems, airflow tests, and cleanliness class recovery tests for all hot cell chambers will prove the grade B classification for the Material Outlet Chamber.

The tests must be periodically repeated, under normal operating conditions, to ensure the maintenance of microbiological contamination levels.

4 CONCLUSION

The microbiological tests confirmed the effectiveness of the cleaning protocols and sanitizers used for the disinfection of the cleanroom and hot cell.

The GMP standards of Anvisa, FDA, and EMA establish similar criteria for microbiological classification of clean areas, with EMA adopting a broader approach considering potential sampling limitations, as long as they are justified.

The tests performed quantitatively demonstrated that the DC, RIC, CIC, and Solid Waste chambers met the regulatory microbiological requirements for aseptic radiopharmaceuticals preparations.

The tests performed for the Material Output Chamber indicate partial compliance with the microbiological requirements for its classification as grade B. Due to the limitations of the active air sample, additional testing is required to confirm the classification of this chamber, mitigating the risk of microbiological contamination.

REFERENCES

[1] ELLIOTT, A. T.; HILDITCH, T. E., MURRAY, T.; MCNULTY, H. The design and construction of a central radiopharmacy. **Nuclear medicine communications**, v. 14, n. 4, p. 328-334, 1993.

[2] SAHA, G. B. Radiopharmaceuticals and Methods of Radiolabeling. *In*: F**undamentals of nuclear pharmacy**. Cleveland: The Cleveland Clinic Foundation, 1998. P. 79-110. ISBN 0-387-40360-4.

[3] VIDAL, A. *et al*. ARRONAX Cyclotron: Setting up of In-House Hospital Radiopharmacy. **BioMed Research International**, v. 2020, n. 1, p. 1572841, 2020.

[4] ANWAR, A. R., *et al*. Design of GMP compliance radiopharmaceutical production facility in MINT. **No. IAEA-CN-130**, Vienna, 2005.

[5] SANTOS, O. R. *et al*. Radiopharmaceuticals Industry Based On the Brazilian Regulations. **FABAD Journal of Pharmaceutical Sciences**, v. 33, n. 4, p. 205, 2008.

[6] OLIVEIRA, S. R.; LEÃO, C. A. História da radiofarmácia e as implicações da Emenda Constitucional n. 49. **Revista Brasileira de Ciências Farmacêuticas**, v. 44, p. 377-382, 2008.

[7] BRASIL - National Health Surveillance Agency of the Ministry of Health, 2022. **Resolution of the collegiate board** (RDC) No. 658. Available at: https://www.in.gov.br/en/web/dou/-/resolucao-rdc-n-658-de-30-de-marco-de-2022-389846242

[8] CALIXTO, J. B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). **Brazilian Journal of medical and Biological research**, v. 33, p. 179-189, 2000.

[9] PATEL, K. T.; CHOTAI, N. P. Pharmaceutical GMP: past, present, and future–a review. **Die Pharmazie-An International Journal of Pharmaceutical Sciences**, v. 63, n. 4, p. 251-255, 2008.

[10] CAMPOS, F. E.; ARAÚJO, E. B. Air system in the hot cell for injectable radiopharmaceutical production: requirements for personnel and environment safety and protection of the product. **INAC: International Nuclear Atlantic Conference Innovations in nuclear technology for a sustainable future**. Brazil, 2009.

[11] BORRELLI, R. A. Functional components for a design strategy: hot cell shielding in the high reliability safeguards methodology. **Nuclear Engineering and Design**, v. 305, p. 18-27, 2016.

[12] TODDLE, S. *et al*. Guidance on validation and qualification of processes and operations involving radiopharmaceuticals. **EJNMMI radiopharmacy and chemistry**, v. 2, p. 1-29, 2017.

[13] YANG, Z. *et al*. Field test of pharmaceutical cleanroom cleanliness subject to multiple disturbance factors. **Journal of Building Engineering**, v. 42, p. 103083, 2021.

[14] DAMASO, G.; DENOYA, C.; QUELLE, D. Controle Microbiano e Monitoramento em Salas Limpas de Processamento Asséptico. Sociedade Brasileira de Controle de Contaminação, p. 38-45, 2016.

[15] WHYTE, W. Operating a Cleanroom: Contamination Control. *In*: **Cleanroom technology: fundamentals of design, testing and operation**. England: John Wiley & Sons, 2010. p 177-191. ISBN 0 471 86842 6.

[16] HU, S.; SHIUE, A. Validation and application of the personnel factor for the garment used in cleanrooms. **Building and Environment**, v. 97, p. 88-95, 2016.

[17] DEN, W. *et al*. Airborne molecular contamination: Recent developments in the understanding and minimization for advanced semiconductor device manufacturing. **ECS Journal of Solid State Science and Technology**, v. 9, n. 6, p. 064003, 2020.

[18] ELSINGA, P. *et al*. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. **European journal of nuclear medicine and molecular imaging**, v. 37, p. 1049-1062, 2010.

[19] BRASIL - National Health Surveillance Agency of the Ministry of Health, 2019. **Normative Instruction** (IN) No. 35. Available at: https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-35-de-21-de-agosto-de-2019-211914062

[20] EU GMP Annex 1: Manufacture of Sterile Medicinal Products. **European Commission**: Brussels, Belgium, 2022.

[21] FDA: Sterile Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice. **U.S. Food and Drug Administration,** 2004. Available at: https://www.fda.gov/media/71026/download

[22] ISO, E. N. 14644-1 Cleanrooms and Associated Controlled Environments—Part 1: Classification of Air Cleanliness. **International Standards Organization**, 2015. Available at: https://www.iso.org/standard/53394.html .

[23] UTESCHER, C. L A. *et al*. Microbiological monitoring of cleanrooms in development of vaccines. **Brazilian Journal of Microbiology**, v. 38, p. 710-716, 2007

[24] MILLER, M. J. *et al*. Evaluation of the BioVigilant IMD-A, a novel optical spectroscopy technology for the continuous and real-time environmental monitoring of viable and nonviable particles. Part I. Review of the technology and comparative studies with conventional methods. **PDA J. Pharm. Sci. Technol**, v. 63, n. 3, p. 244-257, 2009.