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EFFICIANCY OF HYDROGEN PEROXIDE FOR CLEANING PRODUCTION AREAS AND EQUIPMENTS IN THE RADIOPHARMACEUTICAL PRODUCTION

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ABSTRACT

A great challenge in the radiopharmaceuticals production is to fulfill the Good Manufacturing Practices (GMPs), involving the validation of process and of all supporting activities such as cleaning and sanitization. The increasingly strict requirements for quality assurance system, with several norms and normative resolutions has led to a constant concern with programs and cleaning validation in pharmaceutical production. The main goal of GMP is to reduce risks inherent to pharmaceutical production, that is to reduce product contamination with microorganisms and cross-contamination. The basic requirements to prevent contamination is the development and implementation of efficient cleaning programs. In the case of cleanrooms for the production of injectable radiopharmaceuticals, the requirement for cleaning programs is evidently higher due to the characteristics of these areas with hot cells for radioactive materials, where sterile radiopharmaceuticals are manipulated and distributed before administration to patients just after minutes or hours of its preparation. In the Radiopharmacy Department at IPEN it was established a cleaning program for cleanrooms and hot cells using a hydrogen peroxide solution (20% proxitane alfa). The objective of this work was to assess effectiveness of this cleaning agent in reducing and/or eliminating microbial load in the cleanrooms and equipment to acceptable levels in accordance with the current legislation. The analysis was conducted using results of the environmental monitoring program with and settling contact plates in cleanrooms after the cleaning procedures. Furthermore, it was possible to evaluate the action of the sanitizing agent on the microbial population on the surface of equipment and cleanrooms. It was also evaluated the best way to accomplish the cleaning program considering the dosimetric factor in each production process, as the main concern of pharmaceutical companies is the microbiological contamination, in the case of radiopharmaceutical, radiological contamination is relevant. Preliminary studies indicated that the use of the sanitizer **proxitane alfa** is effective in removing viable and non-viable particles in cleanrooms, and it is compatible with the materials, including hot cells and equipments for production. The cleaning process with this sanitizer can be performed quickly, just before the production, enabling the production of radiopharmaceuticals, particularly the ultra-short half-life ones, and does not leave any residue after use.

1. INTRODUCTION

Nowadays, the cleaning of production areas and equipments are acquiring a growing importance in pharmaceutical industry. Good Manufacturing Procedures (GMP) standards take cleaning as a major factor to ensure the quality of the products. In most steps of a

radiopharmaceutical production there are cleaning procedures enrolled, from fractioning of raw material to filling.

Current GMP has guided pharmaceutical industries around the world in regard to cleaning requirements. For example, in Brazil, RDC 17 (*Resolução da diretoria colegiada #17*), a resolution from Brazilian Health Surveillance Agency, rules about sanitizing and hygiene activities, such us personal cares, installations, equipments, utensils, materials and recipients cleaning, sanitizing and cleaning products and any other aspect that could represent a contamination source to the pharmaceutical product [1].

Production areas of sterile products are classified according to environmental conditions and are called "cleanrooms" [2, 3]. In a fludeoxyglucose (F-18) production area, the cleaning requirements have to take in account the special characteristics of these radioactive products, since there is a sterile production and filling inside hot cells, which confers radiation protection. Contamination sources must be eliminated by an embracing cleaning and sanitizing program, approved by the institution Quality Assurance Department [4]. These areas should be frequently monitored as for resistant microorganisms, by sampling methods such as settle plates, air and superficial sampling (swabs and contact plates) [5].

In the Radiopharmacy Department of IPEN (*Instituto de Pesquisas Energéticas e Nucleares*), it was established cleanrooms cleaning programs to the radiopharmaceutical production areas with Proxitane alpha ® 20%, a hydrogen peroxide based solution. The objective of this work was to evaluate if this cleaning product is efficient in reducing and/or eliminating microorganisms load in equipments and cleanrooms to acceptable levels, according to actual legislation [1]. This evaluation was performed by environmental monitoring (viable and non-viable particles, contact and settle plates) performed in cleanrooms after cleaning procedures.

2. EXPERIMENTAL

2.1. Cleaning method

The cleaning method applied in the cleanroom was the manual procedure, utilizing MOPs and Proxitane – alpha 20% sanitizing solution. All materials were previously autoclaved.

Cleanroom always began in the ceiling, followed by fixtures and walls, glasses, equipments, doors, tables and finally the floor.

2.2. Sanitizing solution

The sanitizing solution was prepared with Proxitane alpha (Tech Desinfection®), which is composed of peracetic acid, hydrogen peroxide, acetic acid and water. This product was diluted to a 20% solution for equipments and cleanrooms cleaning, following manufacture's instruction.

After dilution, the solution was filtered with a 0.22 μm sterilizing filter.

2.3. Cleaning frequency

In the Radiopharmacy Department of IPEN, the area cleaning occurs weekly, while the hot cells and equipments cleaning is daily, just before production starts. For this study, the cleanroom cleaning of fludeoxyglucose (F-18) production area occurred in 4 different days and it was evaluated the cleaning method, and consequently, the sanitizing solution spectrum.

2.4. Enviromental monitoring procedures

The following environmental monitoring procedures were performed:

Air sampling:

a) Passive method- Settle plates: 99 mm plates containing TSA agar and Sabouraud 2% agar were exposed for 1 hour in strategic spots.
b) Active method: An air sampler, with 100L/min suction capacity was used. Also it was evaluated its impaction against a surface collection (sieve impactor). TSA agar was used in this test.

Surface monitoring: It was applied the direct contact method by the use of 55 mm contact plates, containing TSA agar (Rodac ®).

Air particles monitoring: Air suspended particles were counted. In this study 0.5 µm and 5.0 µm diameter particles were evaluated. Air monitoring was performance 1 hour after cleanroom cleaning. Values were compared with regulatory agencies standards, in rest condition.

2.5. Monitoring spots.

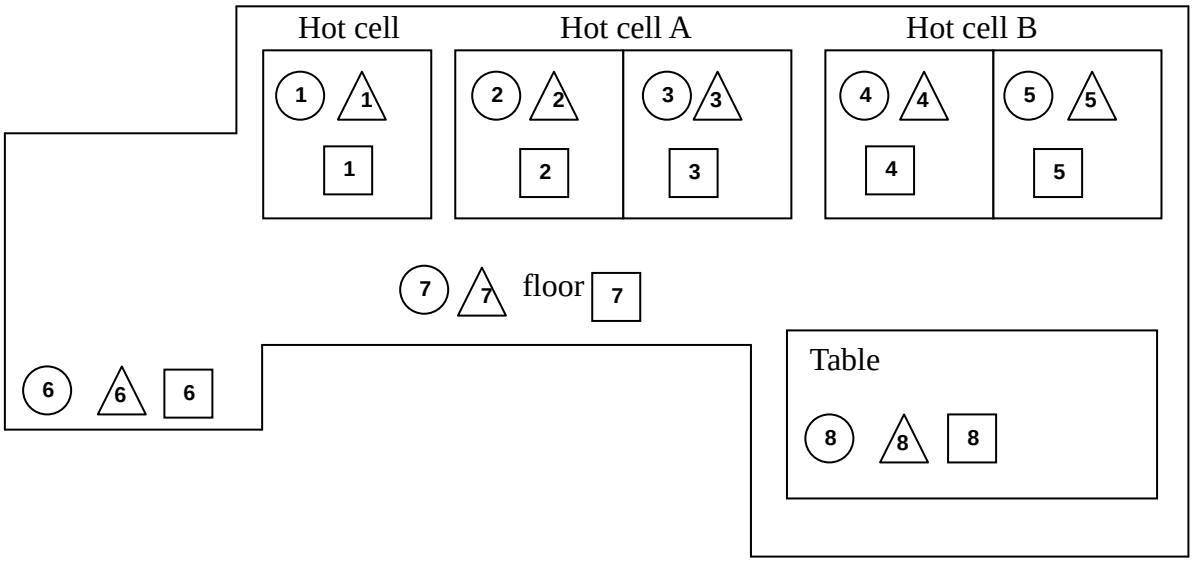

Figure 1: Monitoring spots in the fludoexyglucose (F-18) production cleanroom: ◯ Air sampling; △ Surface monitoring; □ Air particles monitoring.

3. RESULTS AND DISCUSSION

Table 1: Environmental monitoring after cleanroom cleaning process (1st cleaning procedure)

Area	ISO 14644-1	Results Air sample cfu/m^3	Limits [a] Air sample cfu/m^3	Results Contact plates (diam 55nm) cfu/plate	Limits [a] Contact plates (diam 55nm) cfu/plate	Results Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB	Limits [a] Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB
1	ISO 5	2	<1	Zero	<1	zero/zero	<1/<1
2	ISO 7	4	100	Zero	25	zero/2	50/50
3	ISO 7	Zero	100	1	25	Zero/3	50/50
4	ISO 7	2	100	>25	25	zero/zero	50/50
5	ISO 7	1	100	Zero	25	zero/3	50/50
6	ISO 8	8	200	Zero	50	2/zero	100/100
7	ISO 8	16	200	Zero	50	1/zero	100/100
8	ISO 8	12	200	1	50	zero/3	100/100

[a] Recommended limits for microbial contamination. These are average values
[b] Individual settle plates may be exposed for less than 4 hours.

Table 2: Airborne particle monitoring systems

Area	ISO 14644-1	Maximum permitted number of particle per m^3 equal to or greater than the tabulated size AT REST		Results AT REST	
		0.5 µm	5.0 µm	0.5 µm	5.0 µm
1	ISO 5	3,520	20	190,505	247
2	ISO 7	352,000	2900	101,992	341
3	ISO 7	352,000	2900	499,383	412
4	ISO 7	352,000	2900	67,626	82
5	ISO 7	352,000	2900	178,524	318
6	ISO 8	3,520,000	29.000	20,531	706
7	ISO 8	3,520,000	29.000	20,305	636
8	ISO 8	3,520,000	29.000	25,155	282

Table 3: Environmental monitoring after cleanroom process (2nd cleaning procedure)

Sample	ISO 14644-1	Results Air sample cfu/m^3	Limits [a] Air sample cfu/m^3	Results Contact plates (diam 55nm) cfu/plate	Limits [a] Contact plates (diam 55nm) cfu/plate	Results Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB	Limits [a] Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB
1	ISO 5	zero	<1	zero	<1	zero/zero	<1/<1
2	ISO 7	zero	100	zero	25	zero/zero	50/50
3	ISO 7	zero	100	zero	25	zero/zero	50/50
4	ISO 7	1	100	zero	25	zero/zero	50/50
5	ISO 7	10	100	zero	25	zero/zero	50/50
6	ISO 8	6	200	zero	50	zero/zero	100/100
7	ISO 8	zero	200	zero	50	zero/1	100/100
8	ISO 8	zero	200	zero	50	zero/zero	100/100

[a] Recommended limits for microbial contamination. These are average values
[b] Individual settle plates may be exposed for less than 4 hours.

Tabela 4: Airborne particle monitoring systems

Area	ISO 14644-1	Maximum permitted number of particle per m^3 equal to or greater than the tabulated size AT REST		Results AT REST	
		0.5 µm	5.0 µm	0.5 µm	5.0 µm
1	ISO 5	**3,520**	**20**	506	zero
2	ISO 7	**352,000**	**2,900**	10,312	zero
3	ISO 7	**352,000**	**2,900**	7,841	318
4	ISO 7	**352,000**	**2,900**	46,229	12
5	ISO 7	**352,000**	**2,900**	9,865	94
6	ISO 8	**3,520,000**	**29,000**	92,217	18
7	ISO 8	**3,520,000**	**29,000**	78,929	294
8	ISO 8	**3,520,000**	**29,000**	73,053	235

Table 5: Environmental monitoring after cleanroom process (3rd cleaning procedure)

Sample	ISO 14644-1	Results Air sample cfu/m^3	Limits [a] Air sample cfu/m^3	Results Contact plates (diam 55nm) cfu/plate	Limits [a] Contact plates (diam 55nm) cfu/plate	Results Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB	Limits [a] Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB
1	ISO 5	zero	**<1**	zero	**<1**	zero/1	**<1/<1**
2	ISO 7	zero	**100**	zero	**25**	zero/zero	**50/50**
3	ISO 7	zero	**100**	zero	**25**	zero/zero	**50/50**
4	ISO 7	zero	**100**	zero	**25**	zero/zero	**50/50**
5	ISO 7	zero	**100**	zero	**25**	zero/zero	**50/50**
6	ISO 8	04	**200**	zero	**50**	zero/zero	**100/100**
7	ISO 8	10	**200**	zero	**50**	zero/1	**100/100**
8	ISO 8	20	**200**	zero	**50**	zero/zero	**100/100**

[a] Recommended limits for microbial contamination. These are average values
[b] Individual settle plates may be exposed for less than 4 hours.

Table 6: Airborne particle monitoring systems

Area	ISO 14644-1	Maximum permitted number of particle per m^3 equal to or greater than the tabulated size AT REST		Results AT REST	
		0.5 µm	5.0 µm	0.5 µm	5.0 µm
1	ISO 5	**3,520**	**20**	647	12
2	ISO 7	**352,000**	**2,900**	3,437	24
3	ISO 7	**352,000**	**2,900**	1,225	153
4	ISO 7	**352,000**	**2,900**	14,702	24
5	ISO 7	**352,000**	**2,900**	2,837	zero
6	ISO 8	**3,520,000**	**29,000**	56,833	1,377
7	ISO 8	**3,520,000**	**29,000**	47,851	1,413
8	ISO 8	**3,520,000**	**29,000**	69,358	1,307

Table 7: Environmental monitoring after cleanroom process (4th cleaning procedure)

Sample	ISO 14644-1	Results Air sample cfu/m^3	Limits [a] Air sample cfu/m^3	Results Contact plates (diam 55nm) cfu/plate	Limits [a] Contact plates (diam 55nm) cfu/plate	Results Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB	Limits [a] Settle plates (diam.90nm) cfu/4 hours [b] TSA/SAB
1	ISO 5	zero	**<1**	zero	**<1**	zero/zero	**<1/<1**
2	ISO 7	zero	**100**	zero	**25**	zero/zero	**50/50**
3	ISO 7	1	**100**	zero	**25**	zero/zero	**50/50**
4	ISO 7	1	**100**	zero	**25**	zero/zero	**50/50**
5	ISO 7	2	**100**	zero	**25**	zero/zero	**50/50**
6	ISO 8	15	**200**	zero	**50**	zero/zero	**100/100**
7	ISO 8	5	**200**	zero	**50**	zero/zero	**100/100**
8	ISO 8	8	**200**	zero	**50**	zero/zero	**100/100**

[a] Recommended limits for microbial contamination. These are average values
[b] Individual settle plates may be exposed for less than 4 hours.

Table 8: Airborne particle monitoring systems

Area	ISO 14644-1	Maximum permitted number of particle per m^3 equal to or greater than the tabulated size AT REST		Results AT REST	
		0.5 µm	5.0 µm	0.5 µm	5.0 µm
1	ISO 5	**3,520**	**20**	zero	zero
2	ISO 7	**352,000**	**2,900**	7,369	12
3	ISO 7	**352,000**	**2,900**	35	zero
4	ISO 7	**352,000**	**2,900**	42,484	zero
5	ISO 7	**352,000**	**2,900**	18,352	zero
6	ISO 8	**3,520,000**	**29,000**	238,456	777
7	ISO 8	**3,520,000**	**29,000**	290,920	953
8	ISO 8	**3,520,000**	**29,000**	245,678	1,413

In the first cleaning procedure (TAB. 1), the point 1 was out of the acceptable values for active air sampling monitoring. Points 1 and 3 of air suspended particles monitoring were also increased. This first cleaning procedure was performance by newly trained staff, and particulate were visibly present. It shows that, initially, the cleaning process was not efficient and demanded new cleaning staff training.

All the following environmental monitoring procedures presented satisfactory values, showing that the cleaning procedure, as proposed and executed, was able to reduce and maintain the acceptable levels of viable and non-viable particles for the cleanroom classification (TAB. 4, 5, 6, 7 and 8). Also, the sanitizing solution employed, Proxitane alpha 20% was able to control microbiological contamination. Hydrogen peroxide has been shown to kill a wide range of microorganisms including bacteria, viruses and fungi. The efficacy of this products with hydrogen peroxide has been repeatedly demonstrated against bacterial endospores, which are the most resistant organisms commonly found on environmental surfaces, so are positioned at the top of the Spaulding classification. These results show that it would be suitable to establish warning limits to microbiological and air suspended particles monitoring. If these limits are exceeded, a rigorous investigation should be performed, evaluating the cleaning processes.

Another important matter is to characterize the contaminating microorganism. This data will provide essential information about the source of contamination and the environmental conditions. Microorganisms isolated in environmental monitoring might also be related with contaminating microorganisms found in media-fill tests, or in the final product sterility test. It

is highly recommended that the environmental monitoring in critical and surroundings areas, as well as personal monitoring, includes the microorganism identification at the level of genus, or species when possible.

4. CONCLUSION

The establishment of a suitable program for detection and identification of microorganism in non-critical and surroundings areas, such as ISO 8 rooms, can help to identify possible contaminating tendencies. It is recommended, at least, that it occurs at frequent intervals, to create a data bank with the local contaminating microorganisms. This information can be useful to prove that the sanitizing and cleaning procedures are efficient. The environmental monitoring process qualification is an excellent opportunity to initiate this data bank and to generate information about the efficiency of cleaning and sanitizing programs regarding to microbiological contamination.

Also, cleaning procedures validation is an important tool to prevent and control cross-contamination in a pharmaceutical industry.

In summary, this study provided subsidies to monitor the cleanroom cleaning procedure and for further cleaning procedures validation. Only after process validation all the cleaning procedures, the sanitizer solution and its capability to be a residue-free product can be certificated and finally, its efficiency in the cleaning procedure applied.

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