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Main Steps for Radiopharmaceuticals Hot Cells Validation in Accordance with GMP Requirements: Methodology and Practical Guide

Fábio Eduardo de Campos[1], Efrain Araujo Perini[1], Carlos Leonel Zapparoli Júnior[1], Wilson Aparecido Parejo Calvo[1] and Valeriia Niowaveinc Starovoitova[2]

1. Nuclear and Energy Research Institute (IPEN), the University of São Paulo, São Paulo, SP 05508-000, Brazil

2. NiowaveInc, 1012 N Walnut St, Lansing, MI 48906, USA

Abstract: The worldwide GMP (Good Manufacturing Practices) guidelines issued for injectable pharmaceuticals globally agree that the vials filling operation must be performed under air cleanliness Grade A. The air cleanliness classifications adopted by the WHO (World Health Organization) define the particle diameter size, the sampling occupancy state and the limit concentration of viable particles. To reach conformity regarding the microbial limits foreseen at the GMP guidelines, a microbiological monitoring program must be stablished for selected sampling points such as active air sampling, passive air sampling (settle plate method), surfaces sampling (contact method), personnel sampling (gloves and clothes), compressed gas, materials and equipment that may interfere and compromise the product microbiological quality. The key elements for a GMP certification are directly related to a qualification and validation program for radiopharmaceutical manufacturers that must be clearly defined and documented by a validation master plan, foreseen by the manufactures Quality Assurance office. This study describes each qualification step and test for DQ (Design Qualification), IQ (Installation Qualification), OQ (Operation Qualification) and PQ (Performance Qualification) that must be carried out and carefully planned when it comes to hot cells and isolator systems in accordance with the GMP requirements foreseen by international regulatory and supervisory bodies.

Key words: Injectable radiopharmaceuticals, GMP, qualifications, regulations.

1. Introduction

Radiopharmaceuticals are known as injectable radioactive pharmaceuticals widely used for internal radiotherapy for cancer and diagnostic imaging for several body-malfunctions and its usage and applications can be found elsewhere, specially into the nuclear medicine field [1-4]. One must be aware that each country national's health surveillance regulatory authority must approve radiopharmaceuticals, before they can be commercialized and used in humans being.

When analyzing injectable pharmaceuticals production environments one must be aware the product may or may not represent contamination risks to the operator. It is not rare the situations in which it is not enough to protect the product against potential contamination from the environment, but the operator must be protected against being contaminated by handling product. In these cases, isolators represent important roles for the production environment. Isolators can be used to avoid such contaminations, its effectiveness must be reliable and several tests are carried on in order to validate its efficacy. It is worth to mention the air tightness test (carried out accordingly to ISO 10648-2) [5], which is one of the most important acceptance tests. Other tests that can be mentioned for the PQ (Performance Qualification) tests and still applied during the periodic qualification process are HEPA (High Efficiency Particulate Air) filters leakage test, air flow, air changes, air velocity

Corresponding author: Fábio Eduardo de Campos, master, research field: nuclear technology and applications in radiopharmacy facilities.

and unidirectional flow uniformity test, lighting intensity test, particle counting test (at rest and during operation states) and, finally, it is necessary to assure operational condition under negative pressure gradient [6].

For a reliable environment, the radiopharmaceutical production must be focused on the operator safety and product protection, this present work alerts the responsibility for hot cells buyers and manufacturers, and also for the current in-use hot cells regarding its technical aspects and operational conditions, especially the current ones, which are already radioactively contaminated and may present potential risks to the environment and to the operator if some of the qualification test parameters are not taken into consideration. Therefore, more than knowing which the requirements for the production environment are, it is better to know how to reach and meet each of them and mainly, how to maintain them into the requirement levels and tolerances foreseen by the guidelines and reference regulations bodies, such as, the FDA (Food and Drug Administration) [7], the rules governing medicinal products in the European Union (EudraLex) [8], the International Pharmacopoeia (issued by the WHO (World Health Organization)) [9] and others.

2. Isolators and Hot Cells Requirements

Isolators concepts and philosophies are similar to a microenvironment design and its applications to laboratories can be found elsewhere [10]. It can be used not only for protecting the product against contamination, but also for protecting the operator against toxic compounds or for both protections (product and operator) simultaneously. Hence the sealed microenvironment idea is to ensure high levels for viable and non-viable particles controlling into it yielding a reliable and aseptic manufacturing process while it also protects the external environment and the operator.

The utilization of isolator technology minimizes human intervention in processing areas and may result in a significant decrease in the risk of microbiological contamination of aseptic manufactured products from the environment. There are many possible designs of isolators and transfer devices. The isolator and the surrounding environment should be designed so that the required air quality for the respective zones can be achieved. Isolators are constructed using puncture resistant materials and preventing air leakage. Transfer devices may vary from a single door to double door designs and fully sealed systems incorporating sterilization equipment. The transfer of processing materials into and out of the unit is one of the greatest potential sources of contamination. In general the area inside the isolator is the zone for high risk manipulations, although it is recognized that unidirectional air flow may not exist in the working area of all such devices. The air classification required for the background environment depends on the design of the isolator and its application. It should be controlled and for aseptic processing it should be at least grade D. Isolators should be introduced only after appropriate validation. Validation should take into account all critical factors of isolator technology, for example the quality of the air inside and outside (background) the isolator, sanitization of the isolator, the transfer process and isolator integrity. Monitoring should be carried out routinely and include frequent leak testing of the isolator and glove/sleeve system.

Therefore a hot cell can be understood as an isolator, though it has special design and assembling techniques in order to provide its correct usage such as an ergonomic operation and the radiation shielding. Also the material handling inside a hot cell (or containment enclosure) requires an “hourly leak rate” which is related to the processing material dangerousness and, consequently, the hot cell or the “containment” classification can be categorized into four classes, as shown in Table 1.

It is worth to mention that the leak test is carried out at FAT (Factory Acceptance Test) or SAT (Site Acceptance Test) steps and if the isolator or hot cell

Table 1 Classification of containment enclosures accordingto their hourly lake rate (adopted from ISO 10648-2 [5]).

Class	Hourly lake rate ($Tf \cdot h^{-1}$)	Example
*1	$\leq 5 \times 10^{-4}$	Containment enclosure with controlled atmosphere under insert gas conditions
*2	$< 2.5 \times 10^{-3}$	Containment enclosure with controlled atmosphere under inert gas conditions or with permanently hazardous atmosphere
3	$< 10^{-2}$	Containment enclosure with permanently hazardous atmosphere
4	$< 10^{-1}$	Containment enclosure with atmosphere which could be hazardous

*The classification of leak tightness required for a particular application under classes 1 and 2 shall be decided by the designer and licensing authorities. Normally, class 1 will be applied for technical reasons when higher gas purity is required.

fails in this test the manufacturer must review the isolator structure, cracks and sealing.

Accordingly to ISO 10648-2, the leak test for isolators can be performed by the pressure change method, which takes into account corrections due to variations and atmospheric pressure. For acceptance test, the starting negative pressure should be four times greater than the working pressure condition. The leak tightness of containment enclosure must comply with the rate of leakage of a class 2 containment enclosure, in accordance with ISO 10648-2 [5] ($Tf < 2.5 \times 10^{-3}$ or < 0.25%).

Once the hot cell enclosure tightness requirement is achieved, the operation requirements now must be fulfilled in terms of negative pressure gradient. The standardization [6] states that for the handling of radioactive or toxic products, the enclosure is required to be at a negative pressure in relation to the room. This under pressure, the only one of such values easily monitored, is expressed in Pascal (Pa) or Decapascal (daPa), and generally ranges from 20 daPa to 50 daPa below room pressure. Its measurement enables a hierarchy of pressure to be maintained when different enclosures are connected.

3. Product Requirements

As a requirement for aseptic products manufacturing, the clean room in which operations are conducted has its air cleanliness according to the criticality of the environment. Each manufacturing process requires an appropriate environmental "in operation" condition to minimize the microbiological contamination risk, and other potential contamination such as product particles or particles from another used materials. It is interesting to highlight when considering "in operation" test for injectable radiopharmaceutical production hot cells. First of all, the fact that a hot cell does not allow the presence of the operator inside clearly avoids the primary contamination source, indeed GMP (Good Manufacturing Practices) requirements [11] shows that between 30,000 and 40,000 of human-being skin cells fall off every hour. Furthermore to keep the particle counting levels as required is not an easy task if the hot cell is not well designed; this task is related not only to the hot cell design aspects but also to the devices placed into it for the production processes such as crimpers, decrimpers, door opening and closing systems, and so many other devices required for a GMP certified production.

Some short half-life radiopharmaceuticals are usually injected to patients right after the manufacturing process and for this reason the quality control approving tests results are eventually post administrative, such as, sterility test, radionuclide purity test and others to its release and even to its injection. Then, the establishment of regulations and specifications to ensure a reliable radiopharmaceutical production is extremely required, especially for the "in operation" condition that is worldwide foreseen in the EudraLex GMP guidelines [12], such as:

- Grade A is equivalent to ISO class 4.8 "at rest" and to ISO class 5 "in operation". Grade A is equivalent to ISO class 5 for particles diameter size ≥ 0.5 μm (until 3,520 particles per m^3). However, when counting particles diameter size ≥ 5.0 μm Grade A is

equivalent to ISO class 4.8 once it tolerates maximum of 20 particles while ISO class 5 tolerates maximum of 29 particles. Thus Grade A is called ISO class 5 "in operation" for particles size ≥ 0.5 μm and ISO class 4.8 "at rest" for particles size ≥ 5.0 μm. Still regarding Grade A, the GMP guidelines stablish it as a "high operational risk zone for filling and aseptic connections. Normally those operations must be carried out under unidirectional flow conditions" [12];

- Grade B is equivalent to ISO class 5 "at rest" and to ISO class 7 "in operation" states for both particle diameter sizes. The GMP guidelines stablish that grade B is applied to "grade A surrounding area for preparations and aseptic fillings" [12];
- Grade C is equivalent to ISO class 7 "at rest" and to ISO class 8 "in operation" states for both particle diameter sizes;
- For grade D it is only defined the number of particles "at rest" state condition and together with the grade C, they are referred to clean areas where less critical processes are carried out for sterile products manufacturing.
- For a better understanding the EudraLex [12] brings Table 2 for the particle size and air cleanliness classification grades.

In order to face all the GMP requirements to the product and to the manufacturing environmental acceptance, it is required a PQ protocol to be stablished. It is important to mention the qualification process has its basis on the GMP guidelines and it must be developed based on its EudraLex [12].

The qualification process consists of several tests which are divided into small groups for a better task management and sequencing. Each qualification test must be associated to a test protocol where the test procedures and the acceptance criteria are pre-defined. The protocols must be filled out with each test result and the results must be confronted to the test acceptance criteria. After filling out the test protocol with the results the responsible for the test execution must sign the report. A representative counterpart (the hot cell buyer) also must sign the report confirming the tests methodology and results. During the test execution the reference documents must be available for verifications. The qualification can only be concluded when all the protocols are filled out and all the results are in accordance with the acceptance criteria stablished at the protocol.

4. Qualification Tests and Acceptance Criteria

When a radiopharmaceutical manufacturer starts a hot cell specification design, such as by developing the hot cell's URS (User Requirement Specifications), one must be aware of each step and responsibilities are undertaken within this hot cell purchasing process until its conclusion, which means, until the hot cell is qualified according to the GMP requirements. Fig. 1 brings a box-diagram that better illustrates this URS, design and qualifications steps.

When it comes to the hot cell qualification tests, either the hot cell manufacturer or the buyer must be aware of each test requirement to meet all the safety standards regarding to the operator protection and to the product protection, once an injectable radiopharmaceutical is being handled inside it. The

Table 2 The maximum allowed airbome particle concentration for each grade.

	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
	At rest		In operation	
Grade	0.5 μm	5.0 μm	0.5 μm	5.0 μm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Source: adopted from EudraLex [12].

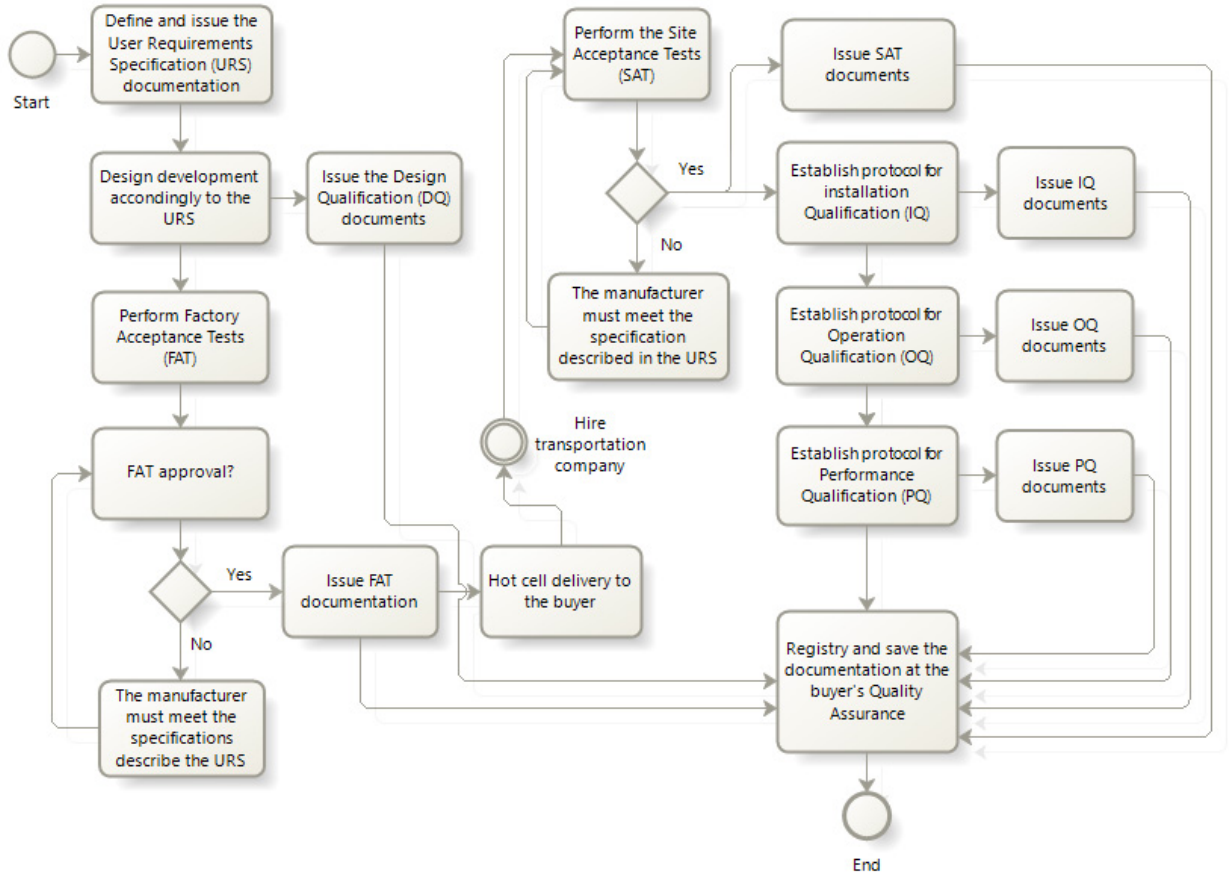

Fig. 1 Box-diagram for hot cell design, purchase and respective qualifications.

following tests are proposed in order to fulfill the hot cell qualification criteria for radiopharmaceuticals production. Each of the following tests has its protocol and the respective standardization reference, stablished as criterion.

4.1 Verification of Filter Integrity (Filter Leakage) for General HEPA Filter and for Unidirectional Flow Units

It is worth to highlight that hot cells require a high efficiency filtering system, either in the air intake (to guarantee the air quality into the hot cell), or in the air outflow (to avoid environmental radiologic contamination). As established by the standard ISO 14644-3 [13], this test is carried out to confirm if the final filtering system with high efficiency filters is properly installed and sealed to prevent leakage or possible contamination to the working area.

Goal: to ensure the HEPA filtering system is in accordance with the specifications regarding to filter integrity and tightness and also guarantee that this filter system fulfills the user requirements. This test does not verify the filtering system efficiency.

Precondition: for traceability reasons, each installed HEPA filter must be supplied with the manufacturer's integrity test certificate and it must be attached to the qualification report.

Standards references:

- IEST-RP-CC006.2—Institute of Environmental Sciences and Technology [14];
- ISO 14644-3:2005—Clean rooms and associated controlled environments—Part 3: Test methods [13];
- ISO 15767:2009—Unidirectional flow clean-air devices—Requirements and test methods [15].

Acceptance criteria:

The filters can be tested following the uranine test

or the DOP (Dispersed Oil Particulate) test. The uranine test has efficiency of 99.98% (the uranine particles dimension is 0.15-0.18 μm); while the DOP test has efficiency of 99.993% (the DOP particles dimension is 0.3 μm). Despite the minor difference in both efficiencies (the DOP test efficiency is higher because the DOP particles are bigger) the filter efficiency remains the same for both tests:

- The upstream aerosol concentration must lay between 20-100 μg/L and the filter leakage level must be ≤ 0.01% of the upstream;
- Filter repair limits—total repair area must be ≤ 3% of the face area of the filter and the smallest dimension of any repair must be ≤ 3.8 cm;
- Filter pressure drop—defined by the manufacturer (measured in Pa).

4.2 Verification Filter One-Way Flow Speed (with Unidirectional Flow Only)

Goal: to check the filter downstream air velocity.

Precondition: the filter integrity test must be done and approved according to the test acceptance conditions.

Standards references:

- ISO 15767:2009—Unidirectional flow clean-air devices—Requirements and test methods [15].

Acceptance criteria:

- The downstream air velocity must be within 0.36 m/s and 0.54 m/s;
- The air flow uniformity velocity deviation must be ≤ 15 %.

4.3 Air Change or Air Change Rate Verification

Goal: to check the air change inside a chamber, room or any controlled environment.

Precondition: all equipment must be operating.

Standards references:

- AABC (Associated Air Balance Council)—Test & Balance Procedures [16];
- ANSI/ASHRAE 111-2008 (RA 2017)—Measurement, Testing, Adjusting and Balancing of Building HVAC Systems [17];
- AMCA 203-90—Air Movement and Control Association International, Inc. [18];
- ISO 14644-3:2005—Clean rooms and associated controlled environments—Part 3: Test methods [13];
- ISO 15767:2009—Unidirectional flow clean-air devices—Requirements and test methods [15].

Acceptance criteria:

(a) The air change rate must be ≥ 20 air changes per hour (20 ac/h).

4.4 Lighting Intensity Verification

It is worth to highlight that, although this verification test is not mandatory, it should be considered during the (factory) acceptance tests because the lighting operating conditions of a hot cell have at least two constraints to consider. First, the hot cells operators need to handle the materials using tele-pliers and check the inner operations through lead glasses windows, which limit the vision by its density and color. Second, it must be considered that minimal lighting intensity may vary from each operator, once visual condition is physiological and it is related to each person.

When searching about lighting intensity guidelines for hot cells, there is no specific study for hot cells, except for one IAEA Safety Series publication [19]. It must be considered this publication is dated from 1981 and the search for an updated study is still required. Thus the only reference we could use is the guidelines [20], which adopts 500 lux for pharmaceutical productions, although it does not consider the lead glass windows, used in the hot cells. Therefore, if one considers the standard adopted by the hot cells manufactures as “around 1,000 lux on the work surface”, it seems to be very reasonable.

4.5 Non-viable Particles Counts Verifications (at Rest)

Goal: to ensure the non-viable airborne particle concentration of 0.5 μm and 5.0 μm of the tested environment at “at-rest” occupancy state is below the

allowable particle concentration according to the designed ISO class.

Precondition: the clean room or controlled environment must be previously cleaned. The test must be performed with the ventilation system in operation. All the internal equipment (filling systems, crimpers, etc.) must be turned on and neither tele-pliers nor tongs should be maneuvered.

Standards references:

- ISO 14644-1:2015—Clean rooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration [21];
- ISO 14644-3:2005—Clean rooms and associated controlled environments—Part 3: Test methods [13];
- Guidance PET drug products to Good Manufacturing Practices (CGMP-Annex 1) [22].

Acceptance criteria (at rest), based on the FDA [22]:

As shown in Table 1 for particle concentrations and average size:

- Grade A (ISO class 4.8 air cleanliness classification) at one single air sampling location;
- Grade B (ISO class 5 air cleanliness classification) at one single air sampling location;
- Grade C (ISO class 7 air cleanliness classification) at one single air sampling location;
- Grade D (ISO class 8 air cleanliness classification) at one single air sampling location.

4.6 Non-viable Particles Count Verifications (in Operation)

Goal: to ensure the non-viable airborne particle concentration of 0.5 μm and 5.0 μm of the tested environment at "in-operation" occupancy state is below the allowable particle concentration according to the designed ISO class.

Precondition: for this particular verification test applied to hot cells, the "in operation" state will be called as "in-dynamical-operation" state because when it comes to an isolator chamber, the tested environment does not have the presence of the operator inside it. Thus the procedure is to simulate, with non-radioactive materials (using water), all the manufacturing process "in-dynamical-operation", such as pass-through doors opening, internal devices operations (vials crimpers, decrimpers, vials fractionating and filling systems, ionization chambers), materials outtake, waste disposal etc., so those operations will indicate if the tested hot cell can fulfill the requirements for the dynamical-operational state.

Guidelines references:

- ISO 14644-1:2015—Clean rooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration [21];
- ISO 14644-3:2005—Clean rooms and associated controlled environments—Part 3: Test methods [13];
- Guidance PET drug products to Good Manufacturing Practices (CGMP-Annex 1) [22].

Acceptance criteria (in operation), based on the FDA [22]:

(a) The same criteria are used for the non-viable particles counts verifications (at rest).

5. Conclusions

A detailed and critical URS for a new hot cell is the bottom line to a successful validation process to comply with GMP requirements.

One must be aware of each design and manufacturing step of an isolator-like equipment or hot cell, starting at the DQ (Design Qualification), then the upcoming FAT (Factory Acceptance Test), after the equipment delivery and installations the buyer must require the SAT (Site Acceptance Test), then the IQ (Installation Qualification), the OQ (Operational Qualification), and the PQ. The analyses of the results obtained during the PQ tests and the evaluation of the conformities (and non-conformities) are the last step for the complete equipment acceptance, which must be under the responsibility of the manufacturer, but also under the buyer Quality Assurance.

Finally, it is worth to mention that the buyer must be aware about the validation and acceptance tests

methodology and instrumentation, once the manufactures may hide some features or criteria in order to show the tested equipment completely approved by the "company" tests, and sometimes the "company tests" has not the same criteria as the GMP requirements and criteria to ensure safety and security to operators, surrounding environment and finally to the patient who will be injected with the radiopharmaceutical produced in these isolators and hot cells.

This study shows the relevance of the primary steps toward successful acceptance criteria aiming the GMP qualification of hot cells and isolators. It also brings international references standards and guidelines to support information to users and manufactures along with orientations toward acceptance and qualification tests. The next studies on radiopharmaceuticals hot cells certification in accordance with GMP requirements will bring practical qualification tests results and discussions (set up guide), and the final study will conclude with a microbiology growth comparison according to the hot cell internal chamber stainless steel surface finishing.

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