Key factors on the installation and validation of a new production line for infusion bags

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Abstract

This main goal of this work is to identify and discuss the main focus points on the instalation and validation of a new commercial pharmaceutical line for the manufacture of terminally sterilized infusion bags. This was achieved through a close monitoring and participation in the initial installation and qualification stages carried out by the engineering, validation, quality control and production departments. A practical and functional-orientated assessment of the main factors that influence the outcome of the new manufacturing department's validation stage was developed. An integrated description of the main focal points that contribute to the overall prevention of contamination development in the department and to the safety of the final product is provided. Specifically, the main steps involved in the installation stage, with a tight focus on the structure, equipment and utilities installed to support the department, as well as in the qualification stage and all the test runs and validation protocols implemented are explained and their relation and connection with the overall success of the validation is demonstrated. With the completion of this work, conditions have been created for an efficient transition to the routine production stage, in compliance with the regulators' requirement, allowing for the continuous monitoring and improvement of the process throughout its lifecycle.

Keywords: Validation - Installation - Qualification - Quality - Safety.

API - Active Pharmaceutical Ingredient
CIP - Clean-in-Place
EM - Environmental Monitoring
EU GMP - European Good Manufacturing Practices
FDA - United States Food and Drug Administration
HEPA - High Efficiency Particulate Arrestance
HVAC - Heating, Ventilation and Air Conditioning
PS - Pure Steam
SAL - Sterility Assurance Level
SIP - Sterilization-in-Place
SOP - Standard Operating Procedure
USP - United States Pharmacopeia
WFI - Water for Injectables

1. Introduction

Validation is an integral part of quality assurance. It involves the systematic study of systems, facilities and processes with the goal of determining whether they perform their intended functions in an adequate and consist manner. A validated operation is one which has been demonstrated to provide a high degree of assurance that uniform batches that meet the required specifications will be produced and has, therefore, been formally approved. [1]

The current approach to process validation, stated in the 2011 FDA's Guidance for Industry on Process Validation [2] clearly emphasizes contemporary concepts and expectations for pharmaceutical manufacturing. The manufacturers should have great confidence that the performance of the process will consistently produce APIs and drug products meeting expected attributes.

This guidance describes process validation activities in three stages:

- **Stage 1: Process Design** - The commercial manufacturing process is defined during this stage based on knowledge gained through development and scale-up activities;
- **Stage 2: Process Qualification** - During this stage, the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing. Besides this, the facility, equipment and support utilities are also evaluated and validated to confirm their compliance with the processes' and the regulators' requirements;
- **Stage 3: Continued Process Verification** - Ongoing assurance is gained during routine production that the process remains in a state of control.

For the particular case of manufacturing areas, as defined in the regulations, separate or defined areas of operation within the pharmaceutical manufacturing environment should be maintained and controlled during production. The design of a given area involves satisfying microbiological and particle criteria, as defined by the equipment, components and products exposed, as well as the operational activities conducted in the area. Clean area control parameters should be supported by both viable and non-viable particulate data obtained during qualification studies. [2], [3]

The initial qualification of pharmaceutical controlled areas includes, in part, an assessment of air quality under as-built and static conditions, but has a main focus on the data generated under dynamic conditions, i.e., simulating the normal operation routine conditions. Besides this, an adequate plan for the monitoring of the environmental conditions of the controlled areas is also essential to assess the conformance with the regulated clean area classification specified for the manufacture of parenteral products.

Considering that the manufacturing line is designed for the production of parenteral products, regulatory authorities require that these products are sterile, which is achieved by means of the terminal sterilization step and is proven by sterility testing according to the pharmacopeia method. [4]

The target of this work is a new manufacturing line that will be built and fully validated to commercially produce terminally sterilized infusion bags. In total, and after the installation and validation of the new line is complete, the new department will include two filling sections, each with its preparation and compounding section, a section for material preparation, as well as an inspection section and several support rooms.

2. Adressing the issue of contamination

It is state of the art that parenteral products must be produced under controlled conditions. Taking into account the regulations and the requirements set by the authorities regarding parenteral preparations and bearing in mind the different sources of contamination cited on the previous section, it is clear that the existence of a multidisciplinary and broad plan for contamination prevention is key. That plan must cover all the areas that end up affecting the production process and/or the final product and, with it, the measures to be taken to prevent contamination must be discriminated.

That plan must be implemented together with an action plan for potential contamination and a sampling and monitoring plan to evaluate the quality of the processes and the success of the contamination prevention program.

In Figure 1 is presented a cause-effect diagram that sums up the most critical areas to be addressed in order to prevent contamination ingress in a production department.

Facilities and Environment

The buildings that contain the production processes and the environment surrounding their location are one of the fundamental aspects to look after when addressing process and product sterility. The location of the facility and the way that the buildings are distributed and constructed can critically influence both the outcome of the processes to be developed on that site and the quality of the product that will be manufactured.

A cleanroom is a room or a specific area with a defined environment control of particulate and microbial contamination, constructed and used in such a way as to reduce the introduction, generation and retention of contaminants within the area. [5] Within a cleanroom, environmental conditions such as temperature, humidity and pressure are controlled and monitored.

For economic, technical and operational reasons, clean zones are often enclosed or surrounded by further zones of lower cleanliness classification. This can allow the zones with the highest cleanliness demands to be reduced to the minimum size. Movement of material and personnel between adjacent clean zones gives rise to the risk of contamination transfer, therefore special attention should be paid to the detailed layout and management of material and personnel flow. [6]

Clean areas for the manufacture of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate level of environmental cleanliness in the operational state to minimize the risks of particulate or microbial contamination of the product or materials being handled.

For the manufacture of sterile pharmaceutical preparations, four grades of clean areas are distinguished as follows (see Figure 2): [7]

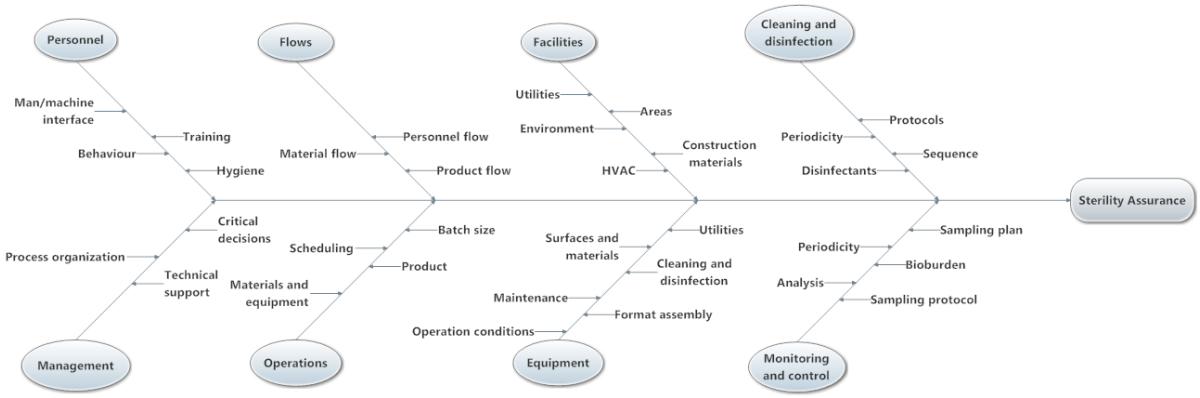

Figure 1: Cause and effect diagram with main parameters to be controlled to avoid contamination in the manufacturing process.

Clean Room Requirements of Japanese and U.S. Guidelines (European Clean Room Grades Provided by Way of Comparison)

Japan		EU	U.S. FDA		EU
Process Areas		Clean Room Grade	Process Areas	Clean Room Grade*	Clean Room Grade
Aseptic Process Areas	Critical Areas	A	Critical Areas	100	A
	–	–	Supporting Clean Process Areas	1,000	–
	Directly Supporting Process Areas	B		10,000	B
Other Supporting Process Areas		C		100,000	C
		D	–	–	D

*Numeric values are specified in [0.5 µm particles/ft³] and apply for the occupancy state "in-operation"; the "at-rest" state is not defined.

Figure 2: Cleanroom classification from USA, EU and Japanese regulatory agencies. Source: Manufacturing Sterile Products to Meet EU and FDA Guidelines, FDA News

The cleanliness grades with the corresponding environmental conditions for the individual manufacturing operations are determined in Annex 1 of the EU GMP Guide (2009). [8] This conditions comply with the recommendations from FDA on the matter and are presented in Figure 3

Cleanliness Grades for the Manufacture of Terminally Sterilized Products

Manufacturing Operations	Room Classes According to the *EU GMP Guide, Annex 1*
• Preparation of solutions and components for subsequent filling • Background environment for blow/fill/seal equipment	• Grade D zone • Manufacturing zone for less critical process steps
• Filling of products for terminal sterilization • Preparation of solutions where the product is at a high or unusual risk of microbial contamination • Preparation and filling of ointments, creams, suspensions and emulsions	• Grade C zone • Manufacturing zone where the operation represents an unusual risk
• Filling of products where the product is at a high or unusual risk of microbial contamination	• Grade A zone • For critical process steps with a high level of risk

Figure 3: Manufacturing operations to perform according with the cleanroom grade for terminally sterilized products. Source: Manufacturing Sterile Products to Meet EU and FDA Guidelines, FDA News

Special considerations have to be made when selecting and installing the cleanrooms. Not only the materials chosen must be adequate to the tasks to be performed and chosen based on the assumption that the cleanliness of the area will be maintained as high as possible, but the layout of the different cleanrooms and the connections between them must also contribute to maintain the area free from contaminants.

Aside from the construction materials, the conditions of the environment must also be controlled in order to guarantee the cleanliness levels required for each clean area. Pharmaceutical HVAC should control airborne contamination and needs to help to ensure the "...purity, identity and quality..." of the product. [9]. The level of air cleanliness (level of airborne particles) depends on: [10]

- Internal activities (control of particle generation into the space from people and processes);
- Particles entering from outside the space, and the ability to keep these external contaminants out of the space;
- Airflow patterns in the unidirectional flow space;
- General room air patterns;
- The quantity of dilution (supply) airflow;
- Cleanliness (quality) of the air being introduced to the space.

The majority of airborne particles are non-viable. A fraction ($< 1\%$) of airborne particles are viable, e.g., bacteria and viruses; however, these can multiply. Viable particles travel with non-viable particles; therefore, controlling the total number of airborne particles also controls the number of viable particles. [11]

The concentration of total airborne particles and microbial contamination within the space is a key measurement of room environmental conditions for pharmaceutical operations. The different regulatory agencies differ on the specifications regarding the concentration limits of airborne particles within clean environments, namely when it comes to the size of particles considered, the state of the cleanroom (if it is operating or "at rest") and the establishment of cleanliness levels. In Figure 4, the acceptance criteria used for the assessment of the level of non-viable particles within clean areas is shown. This criteria was established based on the limits imposed by USP, FDA and the EU GMP guide[12], [7]

Room Classification According to the FDA Aseptic Guidance, USP 34, USP 35 and EU GMP Guide

FDA Aseptic Guidance			USP 34			USP 35		EU GMP Guide				
In Operation			In Operation			In Operation		At Rest			In Operation	
Clean Area Classification	ISO Designation	≥0.5 µm Particles/m³	Class Name	Particles ≥0.5 µm/m³		ISO Class	Particles ≥0.5 µm/m³	Grade	Maximum Permitted Number of Particles per m³ Equal to or Greater than Tabulated Size			
				U.S. Customary					0.5 µm	5 µm	0.5 µm	5 µm
100	5	3,520	M 3.5	100	3,530	5	3,520	A	3,520	20	3,520	20
								B	3,520	29		
1,000	6	35,200	No equivalent			6	35,200	No equivalent				
10,000	7	352,000	M 5.5	10,000	353,000	7	352,000	B			352,000	2,900
								C	352,000	2,900		
100,000	8	3,520,000	M 6.5	100,000	3,530,000	8	3,520,000				3,520,000	29,000
No equivalent								D	3,520,000	29,000	Not defined	Not defined

Figure 4: Non-viable particle limits within clean environments. Source: Manufacturing Sterile Products to Meet EU and FDA Guidelines, FDA News

The ingress of particles and other contaminants from the outside to the interior of the clean areas is contained through air filtration. The air that supplies the department undergoes different filtration steps based on the room it will enter. All the air destined for the filling room passes through a prefilter, an intermediate filter installed in the AHU and terminal high efficiency particulate air (HEPA) filters installed at the entrance of the room. The latter are designed to remove from the air that passes through it a minimum of 99.97% of particles with a size down to 0,3 μm. [13]

For the remaining rooms, where the cleanliness requirements are not as strict, the air passes though a HEPA filter with primary and intermediate filters before entering the room.

The control and management of the particles generated within the process is more troublesome. Overall, there are three main elements that are designed and implemented to access this situation: air change rates, airflow patters and pressurization cascades.

Typically, in non-sterile rooms (Grade C and below), dilution of airborne particles using high room airflow rates is common, relying on adequate mixing of room air with clean air to minimize local areas of high particle concentration. The flow of make-up air introduced in the room is directly related with the particle generation rate of the process, the operations and the equipment present in the cleanroom. [11] Therefore, knowing the particle generation potential within the process is key to calculate and adjust the amount of necessary make-up air to dilute the airborne particles concentration and allow for their continuous removal from the critical areas.

The HVAC system designed for the manufacturing line studied took into consideration a set of factors with the goal of guaranteeing that the product quality was not compromised due to poor air conditioning but also that the personnel and the product safety were maintained. With the implemented system, it is possible to assure that:

1. Cross contamination between the different areas is avoided by proper zoning and pressurization, air locks, and having a flow of air flooding from the cleanest to the less clean space (until exhausted), at predetermined points, with a periodic monitoring of the process;

2. Temperature and humidity are carefully monitored and controlled, avoiding disturbances in the production operation;

3. Records of the pressure, temperature, humidity and filter cleanliness are checked and trended periodically, to assure the right functioning of the system;

Utilities and Equipment

Water is one of the major commodities used by the pharmaceutical industry. It may be present as an excipient, or used for reconstitution of products, during synthesis, during production of the finished product, as a sterilizing agent (in the form of steam) or as a cleaning agent for rinsing vessels, equipment, primary packaging materials, etc. [14].

In the production of terminally sterilized parenteral products, there are two main utilities that are critical for the quality and the safety of the manufactured product: Water for Injection (WFI) and Pure Steam (PS).

WFI is water for the preparation of medicines for parenteral administration when water is used as a vehicle (WFI in bulk) and for dissolving or diluting substances or preparations for parenteral administration before use (sterilized water for injections). [14] WFI is of mandatory use due to its sterility - the absence of pyrogens, endotoxins or other contaminants is essential. Its use applies to the formulation of products, as well as to the final washing of components and equipment used in their manufacture.

Distillation and Reverse Osmosis (RO) filtration are the only acceptable methods listed in the USP

for producing WFI. The system of production and treatment of WFI that supplies the needs of the department is split in two phases: a pretreatment stage where the feedwater is processed through several equipments until compliance with WFI requirements is met, and a treatment step where the WFI is maintained, heated and distributed and where the WFI is directed for the production of PS.

In parallel with the steps taken to assure the low bioburden of the water produced, the design of the production system and the distribution loops was also made considering the impact of the installation, the materials and the components used in the WFI. It is clear that the piping, valves and other elements selected have not only to withstand the sterilization processes used to sanitize the water but also to have virtually no impact on the water's characteristics. Besides this, their installation and placement has to be done to reduce to a minimum the risk of ingress of contamination in the water systems.

Regarding the quality of the materials that compose the treatment systems and the distribution loops, all product contact steel parts should be of stainless steel AISI 316L or comparable. All other product contact parts such a flexible hoses and gaskets must not be toxic, should not establish any sort of chemical reactions with the water, have to withstand sterilization temperatures up to 125 °C, release no particles and should be wear resisting, anti-aging and non-deformable.

All the welds that exist in the system should also be of stainless steel, due to its internal smoothness and resistance to corrosion. Besides this, a special care should be taken throughout the system's design to make sure that gravity drainage is promoted whenever possible, so as to avoid any stopped water for staying in the system.

Material and personnel flows

The overall establishment of both the personnel and the material flows was thought having in mind the details of the department, namely the grades of the cleanrooms, the layout of the department and the logical flow of the process. With this in mind, some general considerations regarding both flows in the department were issued and include:

- The flows aim to cross contamination and environmental contamination, forcing both personnel and material to pass through primary disinfection or gowning areas before entering cleaner areas;

- The use of airlocks as a central part of the flows guarantees the maintenance of the rooms cleanliness conditions. Furthermore, the access to the filling room is obligatorily made through a specific airlock, allowing for an additional steps of gowning or disinfection of personnel and material, respectively, to comply with the requirements of the filling room environment.

- Separate entry and exit routes for personnel and materials were designed, to prevent contamination;

- The number of interventions on the critical areas were designed to be maintained at a minimum. Elements like communication means between the areas and processing instructions were designed to comply with this premise.

Cleaning and disinfection plans

Cleaning and disinfection of surfaces are essential steps for maintaining the cleanliness of pharmaceutical manufacturing operations. The establishment of any production process must include dedicated cleaning and disinfection programs to be applied on a routine basis. This programs must be presented as written procedures (SOPs) and identify the sequence of the cleaning and disinfection, the detergents and disinfectants to use, the frequency of procedures and the appropriate techniques to use. The cleaning programs are constructed with different levels, that separate the number and type of procedures that should be established based on the needs for said procedures (daily, weekly and monthly cleaning and disinfection requirements are differentiated and the requirements for each of these cleaning procedures are specified).

The cleaning and disinfection techniques are extremely important for a successful sanitization since if detergents and disinfectants are not applied in the correct way, areas will not be cleaned effectively and unduly high levels of microbial contamination will remain as the disinfectant will not eliminate all the contaminants. [15] In addition, the cleaning materials used to apply disinfectants and detergents have to be appropriate for the task. The materials must be able to apply an even layer of each agent. For disinfectants and detergents used for floors, surfaces, and walls in sterile manufacturing areas, these must be applied using materials which are cleanroom certified and nonparticle shedding (non-woven and lint-free).

Parallel to the cleaning and disinfection program for the department, it is also necessary to consider and develop adequate plans to clean and sanitize all the pieces of equipment that are in the department, as well as the materials that are introduced into the clean area. When it concerns the materials, before entering the department all carton or styrofoam covers must be removed, since they are particle-generator materials. After they are removed, a disinfection step using either 70 % Isopropanol or a sporicidal disinfectant should be performed.

Regarding the equipment, and in particular the filling machine and the compounding tanks, both elements should have integrated clean-in-place (CIP) and sterilize-in-place (SIP) that are used to perform the cleaning and disinfection of said equipment.

3. Towards product safety

Container/closure system interactions

The prevention of contamination ingress into the clean areas is not sufficient to guarantee that, by the end of the process, the product will be safe for use and risk-free for the patient's health. Aside from contaminants, that have to be actively prevented and dealt with through sterilization processes, there are other issues that can compromise the quality and safety of the final product.

Some of these issues were already discussed and include the quality of the water used for the product's formulation and the terminal sterilization process used to eliminate contaminants in the product. In addition to these topics, and considering the specificity of the product being manufactured, additional references to the interactions between the container and closures used with the product and the additional process implemented to reduce the microbial load of the product prior to the bags' filling were considered pertinent and will be discussed next.

Leachables are chemical entities, both organic and inorganic, that migrate from components of a container closure system or device into a drug product over the course of its shelf-life. Usually they can be found in drug product matrices as complex mixtures at trace levels relative to the active pharmaceutical ingredient (API). [16]

Management of leachables is important to pharmaceutical and biotechnology/biologic product manufacturers and regulatory authorities because certain leachables above specific concentrations can present safety concerns for patients and/or compatibility issues for drug product formulations. [17]

In order to assess the toxicological safety of materials used as containers and closures for the bag system (which is a requirement of the regulatory agencies), an analytical study should be set-up to determine if, and to what extent, the polymer material of the container bags system will release chemical compounds during its contact with the current infusion products (active substances, diluents and solvents).

The study can be designed in two phases, the first being the execution of extractables studies to identify potential leachables compounds. Extractables are chemical entities, both organic and inorganic, that will extract from components of a container closure system or device into solvents under controlled conditions. They are used to identify and quantify potential leachables. [16] With this test, it became possible to determine a worst case migration profile of the bag system and select target compounds to be monitored in the leachables study.

After the target compounds to be screened with the leachables tests were identified, the products to be tested have to be chosen. The criteria to bracket the chosen products concerning their impact on extractables profile usually include: pH, storage temperature, surface contact area and organic content.

For these products, test batches must be performed using the bag system configurations to test and the bags produced should be placed under stability at different storage conditions, so that the leachables assessment was done in multiple stages: immediately after production; at accelerated conditions storage (40 ± 2 °C / < 25 % RH) at 6 months and during long term conditions storage (25 ± 2 °C / 40 % $\pm$ 5 % RH) at 24 months.

Terminal Sterilization

The main requirement for medicinal products that are sterilized in the final container is sterility, which is achieved by means of the terminal sterilization step and is proven by sterility testing according to the pharmacopeia method. [4]

Sterilization is necessary for the complete destruction or removal of all microorganisms (including spore-forming and non-spore-forming bacteria, viruses, fungi and protozoa) that could contaminate the product and that would, consequently, constitute a health hazard. Since the achievement of the absolute state of sterility cannot be demonstrated, the sterility of a pharmaceutical preparation can be defined only in terms of probability. The efficacy of any sterilization process will depend on the nature of the product, the extent and type of any contamination and the conditions under which the final product has been prepared. The requirements for cGMP should be observed throughout all stages of manufacture and sterilization. [18]

Each sterilization method has a particular biological performance capability, that is, a proper capability to kill viable organisms. In the case of terminal sterilized products, the sterilization method selected must achieve a sterility assurance level (SAL) of, at least, 10^{-6}, that is, a 6 log reduction (each log reduction [10^{-1}] represents a 90 % reduction in the microbial population).

The SAL is the probability of a single unit being non-sterile after it has been subjected to sterilization. In microbiology, it is impossible to prove that all organisms have been destroyed as the likelihood of survival of an individual microorganism is never zero, so SAL is used to express the probability of the survival. The minimum value of SAL acceptable for parenteral drugs is 10^{-6}, which means that

the chance to find a non-sterile unit is only 1 in 1.000.000. [19]

The design of the sterilization cycles to be employed to sterilize the bags' loads in the department was done following the so-called overkill approach. The overkill method relies upon the selection of a lethality level known to be adequate to ensure sterilization without routine control over bioburden.

The overkill approach stems from the concept that the sterilization process will inactivate a high micro-biological challenge with an additional safety factor. The microbiological challenge will consist of a biological indicator with a specific number of microorganisms (usually 10^6), and a worst-case assumption is made that the heat resistance of the bioburden is equivalent to that of the biological indicator. Therefore, the cycle conditions established are more severe than those re-quired to inactivate the real product bioburden, and a theoretical spore reduction of 10^{12} is expected to prove the overkill assurance.[20]

The basis for this level is that if the bioburden on an article was one million and all of that bioburden consisted of resistant spores with a D_{121} value of 1 min, then a 10^{-6} probability of a nonsterile unit (PNSU) would be consistently attained. Obviously, this reflects worst-case assumptions regarding both the bioburden level and resistance, which would in every instance be lower in the real-world condition.

Classical sterilization techniques using saturated steam under pressure or hot air are the most reliable and also so most commonly used amongst manufacturers. Other sterilization methods include filtration, ionizing radiation (gamma and electron-beam radiation) and gas sterilization (ethylene oxide, formaldehyde). [18]

4. Validation of a manufacturing department

Once the department is installed and the ancillary equipment that is fixed and used in production is already in place (including the filling machine, the compounding tanks, etc.), the next step on the process validation plan is to qualify the installation of said equipments and guarantee that they are capable of performing consistently the tasks they were designed to perform. All pieces of equipment that will be used or installed in the line have to be previously validated before being used in a production context.

All the pieces of equipment to be validated as covered either by the validation master plan established for the department or an individual validation plan, that will specify all the conditions that must be met and all the challenges that the equipment must pass in order to become apt for use. The standard process qualification protocols applied in a department should include a first stage of installation qualification, where the equipment is evaluated and its installation is conducted and certified, followed by an operational qualification stage where the functioning of the equipment under the desired settings of operation must be demonstrated.

Following the installation of the HVAC system and the HEPA filters that handle and sterilize the air that enters the department it is necessary, both for validation and for routine qualification purposes, to establish the monitoring protocols used to evaluate the state of those elements.

There is a series of specific tests that have to be performed to validate the air handling units, including: calculation of the air changes per hour, measurements of the air velocity, airflow and air patters, measurement of the differential pressure between rooms and, for the HEPA filters, measurement of the pressure drop of the filter and leak tests.

After the installation of the cleanrooms is complete and the ancillary systems that help containing the ingress of contamination are functioning (namely, the HVAC system), the result of said installation and the impact of the routine operations on the sterility levels that has to be assessed.

Monitoring cannot identify and quantify all microbial contaminants present. Furthermore, microbiological monitoring of a cleanroom is technically a semi-quantitative exercise, given the limitations in sampling equipment and the lack of precision of counting methods and limited sample volumes mean that environment monitoring is incapable of providing quantitative information regarding sterility assurance. [21]

However, the real value of a microbiological monitoring program lies in its ability to confirm consistent, high quality environmental conditions at all times. Monitoring programs can detect changes in the contamination recovery rate, that may be indicative of changes in the state of control within the environment, making it possible to act in order to reestablish the normal conditions.

The evaluation of the environmental conditions of the department for the purpose of its initial qualification was performed following a written procedure for environmental monitoring (EM). In this procedure, all the knowledge regarding the layout of the area, the air handling within the department and the operations performed was used to establish a general microbiological sampling plan, that includes the type of sampling to perform, the sampling points and number of measures to perform during the validation of the department and throughout the routine operation. The several stages that go from the design of the EM plan until the trending of the obtained results are summarized in Figure 5

For the purpose of the initial qualification, the sampling procedures of both viable and non-viable

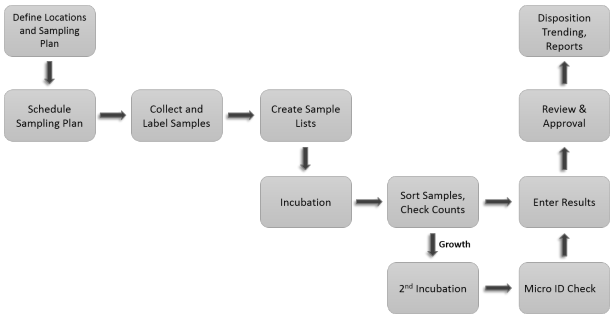

Figure 5: Scheme illustrating the EM process.

particles should be designed to be performed in static and dynamic conditions, following the Environmental Monitoring (EM) plan designed for the initial qualification of the department. For static monitoring, a room must have no activity other than the highly trained sampling operators for generally three hours or greater to be considered in a static condition. A dynamic room is expected to be operating according to routine manufacturing operations and personnel in order to have a representative viable and non-viable particulate reading. [22]

The static monitoring is performed to establish the baseline count of particles in a room, that will represent the optimal conditions and will be used as a reference to determine the decontamination time of the room. On the other hand, monitoring under dynamic conditions is used to routinely monitor points of critical exposure of the product and other reference points in the room, with the presence of the operators and the normal functioning of the production process.

The environmental monitoring plan also defined the type of samples to be gathered, in order for the area monitored to be qualified. Overall, there are two main types of samples that can be considered: air samples and surface samples. In addition, sterile water lines, product ingredients and finished products are randomly selected for sampling. [23]

Regarding the monitoring of airborne particles, there are different protocols used for the monitoring of viable and non-viable particles. For non-viable particles, the sampling is performed using an airborne particle counter, that traces the existence of particulates such as dust, skin and other contaminates suspended in the room air. The volume of air sampled to perform the particle count was different whether initial qualification or routine monitoring was being performed: for initial qualification, 1 m^3 of air was sampled in the locations classified as Grade B and Grade A air supply (in the department, only the inside of the filling machine has this classification), whereas for grades C and D, the volume to collect will be 1 ft^3. In all cases, the sampling results are the average of three consecutive reads.

For the monitoring of viable particles, the distinction between sampling for airborne particles and surface contaminant particles can be made. There are two main procedures that are implemented to perform the sampling of airborne viable particles: active air sampling using air samplers and passive air sampling using settling plates.

On the case of surface particle monitoring, two cases can be considered. To determine the environmental conditions of the department regarding surface contamination - this is a measurement of the efficacy of the cleaning and disinfection procedures - RODAC or surface plates are used. RODAC plates are also the sampling method used when personnel gowning has to be monitored. There is a standard protocol for sampling, regarding the sampling locations and order of sampling, that must be followed to access that the gowning procedures are well performed and that no contamination is being introduced in the area via gowning. There is also another protocol of surface sampling that can be used to access mainly the effectiveness of the cleaning protocols on equipment and other materials, which is swabbing.

Regarding the validation of the terminal sterilization step, the validation plan of this stage occurs in multiple steps. Firstly, the autoclave used to run the sterilization cycles has to be fully validated to confirm its ability to properly sterilize the loads. In parallel, there is also a need to validate the loads that you intend to sterilize, namely the disposition of the load and the operating parameters, in order to assess the optimal functioning conditions and to assure the final sterility of the product.

The qualification of a sterilization cycle is achieved through heat distribution and heat penetration studies, that demonstrate the uniformity, reproducibility and conformance to specifications of the production sterilization cycle for a specific autoclave.

The studies must be performed in two phases: firstly, empty chamber studies should be conducted, in order to measure the temperature distribution profile within the autoclave, so that hot and cold spots in the sterilizer were detected and the temperature uniformity was verified. Following this study, additional runs with both maximum and minimum loads have to be conducted, to demonstrate the effects of loading on thermal input to the product.

During heat penetration studies, sensors should be placed in the containers at their slowest heating point. The majority of these containers will be located at the slowest heating point in the loading pattern, as determined by the heat distribution studies. The amount of heat delivered to the slowest heating unit of the load will be monitored and

this data will be employed to compute the minimum lethality (F_0) of the process. Once the slowest heating units of the load have been identified, three replicate runs will be performed to verify that the desired minimum process lethality factor can be achieved reproducibly throughout the load. The process is considered acceptable once such consistency in lethality has been adequately established.

For the heat penetration studies, the acceptance criteria already take into consideration not only the lethality factor of the cycle but also the actual capability of the cycle to eliminate bioburden.

Once all the elements regarding heat distribution and heat penetration studies to validate the sterilization cycles are complete, the next stage will be load optimization. This will consist on conducting a series of runs varying the size, distribution and operational parameters used (always within the requirements imposed by the authorities) in order to assess the optimal conditions for sterilization for each type of load to sterilize.

All the tests conducted should be annually repeated, as part of the requalification program effective towards sterilization cycles and sterilizers. On this scenarios, the loads that have been determined to be the worst-case will be the ones assessed and the results of the tests will have to remain in agreement with the ones obtained during the initial qualification stages.

5. Conclusions

Overall, the main objectives outlined for this work were achieved. A deep insight on the more important parameters that have a direct impact on the quality of the final product was presented and the information detailed on this work acts as an important tool to describe, qualify and integrate the different aspects that make the global validation plan of the department.

The current approach to validation recommended by the regulatory authorities clearly emphasize the need for well documented and successful installation and qualification stages of process validation, that allow for an efficient and compliant transition to the routine commercial manufacturing stage.

Once that is done, the implementation of the continuous process verification phase will be made, as a part of the overall validation master plan, reflecting the ideas and criteria established during the initial qualification stages.

Focusing more specifically on the implementation of a continuous monitoring plan, amongst the tools used for continuous verifications, product stability programs, change control processes and the Annual Product Review Process are vehicles used for monitoring and assessing process stability. As a complement to these tools, an approach to sampling with a focus on looking at intra and inter-batch variation of the critical quality attributes (CQA) of the product of the product can also be used to monitor the commercial process stability.

The data obtained during production and revalidation efforts should be trended and analyzed employing statistical process control and process control charts. With it, there is the potential to maintain a constant re-evaluation of the alerts and action limits established during the initial qualification stages, allowing for the constant improvement and control of the process throughout its lifecycle.

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