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Microbial Monitoring in Pharmaceutical Cleanrooms

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Abstract

Pharmaceutical clean rooms are controlled area in which the concentrations of viable and non-viable airborne particles are controlled to specified limits. The viable monitoring includes microbiological assessment. Viable contamination controlled environments are used for a variety of purposes within the pharmaceutical industry. Different sources are there due to which pharmaceutical controlled environments are getting contaminated but one of the main sources is air born particulate matter. The predictable microbial assessment of the contamination can be done by applying best microbiological approaches, methodologies and historical data of the area used for the manufacturing. This review article outlines the, designs, control measures, risk of

microbiological contamination and quality rationales related to the clean rooms.

Key words: Bio-burden, clean room, contamination, environment**,** particulate matter.

Introduction

At first the requirement of control environment during a work operation was in medicine. During the latter part of the 19th century, physicians learned that microorganisms were responsible for transmission of infections. A great number of these carriers were present in an infected patient. Hospitals were also recognized as a primary source of further infection. Operating rooms were established in which an effort was made to control the spread of infection. The emphasis in the operating room was in sterile conditions or control of biological contamination.

Present clean room operating techniques are the consequences of World War I. The origin and development of clean rooms were also tied to World War II. The aircraft and navigation devices of World War II required many small components with close tolerances. Dusty atmospheres and the general un-cleanliness of a machine shop were soon recognized as a source of much difficulty in quality control and reliability assurance. As a result, controlled assembly areas were constructed, and some semblance of cleanliness established [1].

Guidelines for clean rooms

The guidelines for cleanliness classes of clean room in the United States were published in June 16, 1988 as a FED-STD-209E (Unites States Federal Standard 209 E). As time progressed clean rooms became a fundamental need for maintaining the clean room environments which gave birth to a single standard for clean room classification in the form of ISO 14644. In 1999 ISO 14644-1 and in 2000 ISO 14644-2 guidelines were published by ISO. The publication of these guidelines begins the process of cancellation of the FED-STD-209 E . In 2001 FED-STD-209 E was superseded by ISO 14644-1 and ISO-14644-2 [2].

The design, construction and cleanliness specifications of clean room environments are

maintained as per ISO 14644 series. This series of standard defines the performance of a clean environment with respect to the concentration of total particulate matters per unit of volume [2]. ISO 14644 is the guideline for clean rooms and contains following parts:

ISO 14644-1: Classification of air cleanliness

ISO 14644-2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1

ISO 14644-3: Test Methods

ISO 14644-4: Design, Construction, and Start-up

ISO 14644-5: Operations

ISO 14644-6: Vocabulary

ISO 14644-7: Separative devices (clean air hoods, glove boxes, isolators and mini environments

ISO 14644-8: Classification of airborne molecular contamination

ISO 14644-9: Classification of surface particle cleanliness

ISO 14644-10: Classification of surface cleanliness by chemical concentration

ISO 14644-12: Classification of air cleanliness by nanoscale particle concentration

In pharmaceutical regulatory guidelines for test and specifications of clean room parameters of all the countries regulatory guidelines refer to the ISO 14644 series standard for classification of clean rooms. Clean room may be sterile and non sterile, often it is misunderstood by the professionals working in the pharmaceutical sectors that, if the clean room comply the air particulate matter class as per ISO means it's sterile. ISO classification for the cleanliness class of a clean room is based on the presence of only particles most often there are understood non viable particles. Pharmaceutical manufacturers are concerned with nonviable

particulate contaminations in sterile products. Unlike microbial contamination in which experimental data suggest that human and human mediated activities are the only major source, nonviable particulate matters can occur both from humans and from processing equipments. Studies showed that gowned human slough particulate and microbial contamination at a somewhat consistent rate. However, the relationship between viable and nonviable contamination does not hold for particulates shed by processing equipment. Where equipment is the primary source of particulate matter, the resulting particulates are essentially all nonviable [3, 4].

The fact that if fewer total particulate are present in a clean room, it is likely that air born microorganisms will be present is true only if human activities are the source of particulate matter. It is impossible to distinguish between total particulate contamination generated largely by mechanical operations and total particulates contributed by humans. Thus, it is essential that best environmental monitoring program of pharmaceutical clean room should consist the provision for monitoring of viable and nonviable particle monitoring procedures. The limit of particulate matters in clean rooms as per ISO 14644 is given in Table-1 [3].

Table-1 Airborne total particulate cleanliness classes

ISO class	Particles (>0.5µm/m3)
ISO 5	3520
ISO 6	35,200
ISO 7	352,000
ISO 8	3,520,000

Microbiological limits are also critical issues when evaluating pharmaceutical production clean rooms [5, 6]. The manufacturers are expected to have microbiological standards based on their process historic values. The characterization of the microorganisms also is an expectation,on the basis of criticalities of infection and risk assessment it has to be done.

Suggested initial on the basis of criticalities of infection and risk assessment it has to be done. Suggested initial

microbial contamination recovery rates in aseptic environments have been given in Table-2 [7].

Table -2 Suggested initial microbial contamination recovery rates in aseptic environments [7]

Room classification	Active air sample (%)	Settle plate (9 cm) 4 hours exposure (%)	Contact plates or swabs (%)	Glove or Garments (%)
Isolator/ Closed RABS (ISO 5 or better)	<0.1	<0.1	<0.1	<0.1
ISO 5	<1	1<1	1<1	1<1
ISO 6	<3	<3	<3	<3
ISO 7	<5	<5	<5	<5
ISO 8	<10	<10	<10	<10

Importance of microbiological monitoring in clean rooms

Monitoring of total particulate matter in a controlled environment by using electronic devices on a daily basis does not provide information on the viable content of the environment .The basic limitation of particulate counts is that they measure particles 0.5µm or larger. While airborne microorganisms are not free floating or single cells, they associate with particles of 10-20 µm. Particulates as well as microbial counts in controlled environment vary with the sampling location and the activities being conducted during sampling. Monitoring of the environment for particulate matters (non viable) and microorganisms is an important control function because they are important for compliance with specification limits as particulate matters, total aerobic microbial count, pathogen and sterility for injectables of product which are being manufactured in the environment [3, 8].

The main purpose of controlled clean rooms in the pharmaceutical, medical device and biotechnology, hospital applications is to control 'bio-burden' due to internal operations and due to transport from the air. From a particulate matter point of view, clean rooms in these industries are classified and specified according to the same clean room standards as in other industries. Often it is assumed that the particle concentrations will generally correlate to the concentration of viable counts. This may not always be valid. Hence, the concentration of viable organisms is also directly measured both at the work surfaces and in the air. Clean rooms in these industries must meet separate standards for bio-burden.

The food and drug administration (FDA) has specific requirements and guidelines for bio-burden of various pharmaceutical operations and processes. Similarly, the USP and the European Union's GMP guidelines give specific recommended limits for microbial contamination for each class of room. A clean room that meets the particle concentration requirements, but does not result in the desired level of bio-burden, will clearly be inadequate. One of the major challenges in achieving the specified limit of bio-burden in clean rooms, because it is time consuming. Normally bio-burden assessment involves sampling, passive air sampling by settle plate method and active air sampling by using air sampler incubation and then counting of colonies in the form of colony forming units (CFU). This is lingering process due to which real time monitoring is not possible. Thus, it is not always possible to relate higher microbial count to operating events [8, 9].

The verification and assessment of competence of sterile and non sterile clean room environment are essential requirements. Clean room environment maintaining protocols are designed in such a way that to identify potential sources of contamination. This will help to find out the root cause of failure and to implement the corrective action and preventive actions (CAPA) in order to minimize the clean room contamination , this will also provide information about occupational practices , engineering control measures and sanitization which will help to maintain the area ,acceptability low microbial levels [10].

Types of contamination

Apart from particulate matter, i.e. viable and non viable ions and molecules are the most common types of contaminants examples are people as hair, ciliary fibers, clothes, dust particles, contaminated air, work surfaces, gases, walls and floor materials, fibers, movable equipment parts shavings drives belts, flakes from manufacturing facility paint and rusty pipe work. Water micro organisms grow in water. Equipment not cleaned correctly and left in a clammy condition, spillages not mopped up properly many types of microorganisms are potentially harmful to processes in a clean room environment. Most common contaminants are *Aspergillus brasiliensis, Clostridium difficile, Bacillus cereus, Burkholderia cepacia, Salmonella enteritidis, Escherichia coli, Methicillin-resistant Staphylococcus aureus* (MRSA), *Legionella pneumophila* and *Pseudomonas aeruginosa.*

Effect of contamination

Contamination is a risk to manufacturing process and to the individuals involved. Uncontrolled growth of microorganisms can lead to the failure of product, product recall, yield reduction and consequently leads to loss of company business. Most of the business losses are due to contamination related issues in the industries which ultimately leads the harmful business relation and imposes the loss to industry [10].

Control of contamination

Contamination control is a continuous process in controlled clean rooms used for manufacturing of pharmaceutical products and is equipped with high efficiency particulate air (HEPA) filters also sometimes called high efficiency particulate arresting air filters used in clean rooms for achieving the required ISO cleanliness class. The filter must comply standards of efficiency i. e., an air filter must remove 99.97% of particles that have a size of 0.3 µm and other contamination controlling measures are a personnel movement, fogging of the environment, sanitisation of the area. These measures control the number of particulates present within the

atmosphere and inhibit the growth of microorganisms. Workmen movement also causes contamination. The protective clothing such as helmets, clean room dresses and masks are basic protective items for controlling of contamination in clean rooms [2].

Continual improvement approach for control of contamination

Term "GMP" that is Good Manufacturing Practices is followed in routine through which day to day basis whatever the experience is gained and implemented is known as continual improvement and is abbreviated by (cGMP) that is current good manufacturing practices. Continual improvement outcomes of actions taken for claims, deviations, emergencies and review of standard procedures, change control, risk assessments are useful for maintaining cleanliness in term of control of bio-burden in clean rooms [8, 11].

As workers may be a major source of contamination for microorganisms, endotoxins, non viable particulate matters, and other particulate matters, it is must to minimize workmen intervention which is a major source of contamination in finished goods. Personnel involved in manufacturing of pharmaceutical products in clean room should be educated and trained on standard operating procedures of manufacturing processes and maintenance of clean room environment equipments.

Operational procedures required during manufacturing must be incorporated in the standard operating procedures (SOPs) and their authenticity with respect to the intended use for maintaining a clean room environment should be ensured. Personnel involved in routine activities carried out in clean room should be trained in all the procedures required for manufacturing of pharmaceutical products. As there is a risk associated with the elevated microbial burden, endotoxin , particulate matter during manufacturing . The training should be imparted to all levels of employees and following topics should be emphasized:

- Fundamentals of microbiology
- Endotoxin-Origin, biochemistry and associated risk
- Entry, exit and gowning procedures

- Disinfection and sanitisation practices
- Control of foreign particles
- Material safety data sheet (MSDS) of product to be manufactured or being manufactured
- Sterilization and sterilization techniques
- Prevention of cross contamination
- Factors affecting the clean room environments
- Techniques and measures used for controlling of bio-burden

Precautions for the personnel involved in preparation , filling, batch charging , milling, sifting, sterilization and sealing should follow the below mentioned precautionary measures:

- Ornamental items, rings, wrist watch and cosmetics items must be prohibited.
- Working should be done in such a way that there should me minimum contact ability with wall surfaces and equipments.
- Standard operating procedures (SOPs) and procedures should be followed strictly.

Viable particulate monitoring

Total aerobic microbial count (bacteria, yeast and moulds) in sterile and non sterile clean rooms are direct threats to clean rooms products which are manufactured in the facility. The regulatory agencies for pharmaceutical products, treaties and forums define and provide the action limits and minimum acceptance limits with frequencies of bio-burden monitoring. There is an expectation of the regulatory agencies to have the tighten alert limit, whatever the limit is fixed in respective guideline. They also expect that pharmaceutical manufacturer have to calculate the alert and the action level on the basis of their manufacturing clean room results by using the statistics, there should be a provision in the standard operating procedure to do the periodic reviews for all forms of viable and nonviable particulate matters. Periodic review of results provides a degree of cleanliness in term of bio-burden status of the respective area over the period of monitoring taken an account for review. This is the best tool for data management and trending of results [10, 12].

Test Methodology

Bio-burden monitoring in pharmaceutical clean rooms is generally done by active air sampling and passive air sampling. Active air sampling provides colony forming units (CFU) per unit of meter cube of air (cfu/m^3) while settle plates are exposed for four hours whatever the methodology used for routine monitoring of bio-burden should be validated with respect to growth promotion property of used media, sampling methods, sanitisation practices followed during the study and incubation time and temperature. The advantage of validated methods is that it provides the road map and assurance that the results are aligned to previous validation results and ongoing studies are towards the perfect direction which is always remains the expectation of competent authorities [12].

Alert and Action limit concept

Often the term alert and action limits are used rather than "limits" only. The term limit implies that the product has been impacted by an excursion above that value. The use of alert and action limits does not imply that the product has automatically been impacted and is the generally accepted term. An alert limit is a level that when the microbial count exceeded, indicates a process may have deviated from its specified condition. Alert levels provide a warning, but do not necessarily warrant corrective actions. Action limit is a level that when the microbial count exceeded, indicates a process has deviated from its normal operating range. A response to such an excursion should be taken an account of investigations and corrective action [2,13].

Discussion

The pharmaceutical manufacturing clean rooms should control the contamination effectively from workmen, accommodating service, finished product raw materials and process equipments as well. The clean room designing should be as per latest guidelines and must be built up in such way there should be no loop hole which can contribute the contamination during the production activities. The maintenance of clean room should be done periodically with

covering all possible risk contributing points. It is always expected that the well educated and trained manpower has to be deployed in clean rooms. It is also expected that the well established monitoring methods used in clean rooms can only determine a proportion of the viable micro-organisms present in the clean room air. However, these methods work reasonably well in isolating the micro-organisms most commonly found in clean rooms. The bio-burden data into a specific statistical distribution is less critical than understanding the ranges of bio-burden over time. An important part of this process is a good definition of alert and action limits and understanding what should occur when each is triggered. It's important to find a balance for the specified actions when exceeding alert or action levels. Understanding the regulatory situation as it relates to the microbiology function is never easy. This is true both for the quality oversight of microbiology related issues for microbiology and also for the management charged with overseeing the microbiological functions.

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