APPLICATION NOTE

Cleanrooms, GMPs and ISO classification

MCO-170AICUVH-PE MDF-DU702VX-PE

Recent successes in cell and immune-mediated therapies have accelerated the possibilities for cell-based therapies as a mainstream form of treatment across the globe.[1] As stem cell research and regenerative medicine continue to gain clinical importance, the need for reproducibility, reliability and contamination-free performance of basic laboratory equipment is essential to the quality process.

Laboratories and other settings that work with biologically relevant products require a clean and controlled environment to establish and maintain sample integrity. Contamination events cost both time and resources, leading to significant delays in research, as well as patients' ability to receive therapeutic treatments.[2] One of the most impactful precautions laboratories and clinical manufacturers can take in avoiding contamination is establishing and maintaining a cleanroom that meets Good Manufacturing Practice (GMP) guidelines. GMP regulations apply to all phases of cell collection, processing and storage.[3]

Threats to Cell Culture-Based Product Production

Sterile or aseptic assurance is an important factor for manufacturing living cell/tissue products that cannot be sterilized. Initial cell and biological materials have inherent susceptibility to contamination from microorganisms and viral contaminants.[4] Given that atmospheric air is laden with microparticles of a potentially infectious nature, maintaining a proper cleanroom environment with appropriate equipment minimizes the risk of potential stressors and exposure to contaminants.[5] Cell culture contaminants can originate from other mammalian cells, microbial sources or can be from a non-living source. Any form of these contaminants present in cell cultures can adversely impact results, producing genetic instability, transformation, changes in normal physiological function and changes in viral susceptibility.[6]

Many contaminants can be detected in the early stages of culturing through visual cues, while other contaminants are more inconspicuous and difficult to identify. For example, microbe and mycoplasma contamination can be detected relatively easily, while contamination from viruses is more difficult to detect. In fact, viral contamination often does not present in overall morphological changes; rather, it manifests in DNA changes that cannot be visually discerned.[7] Viral infection can originate from contaminated cell lines, contaminated raw materials or a GMP breakdown in the production and purification process.[8] Primary contamination also presents the risk of cross-contamination to other products, creating an even greater loss of resources and time.

What is a Cleanroom Classification?

A cleanroom is a controlled environment where the concentration of airborne particles like dust, microbes and aerosol particles are controlled. Cleanrooms are maintained and utilized in a manner that minimizes the introduction, generation and retention of particles in the environment. All cleanrooms that meet the GMPs are classified according to the cleanliness level of the air inside them.[9]

Meeting requirements for cleanrooms is one of the most critical issues for laboratories adhering to GMP requirements. The International Organization for Standardization (ISO), a non-governmental body charged with promoting worldwide standards to ensure safe, reliable and high-quality products, developed classifications associated with the levels of cleanroom certification. ISO awards cleanroom designations based on a threshold of allowable particles within a specified area (Table 1).

According GMPs requirements, cleanroom classifications should be carried out according to ISO 14644-1. This ISO classification impacts every cleanroom user in the GMP community.

Who Needs an ISO-Certified Cleanroom?

Many laboratories and clinical facilities need cleanrooms for a variety of reasons. Contaminants or particles in the air greatly impact the process of both testing and manufacturing samples and products. The creation of particles by certain laboratory

equipment can lead to accelerated degradation, contamination and total loss of biologically relevant material.[10]

Out of the top 10 best-selling drugs of 2019, 7 were biologics (Table 2).[11] When manufacturing biological products, it is imperative to acknowledge that any change during the manufacturing process, regardless of how minor, may alter product quality and efficacy.[12] Therefore, within GMP facilities, it is important to classify your clean areas accurately. One of the first steps in this process is implementing cleanroom certified furniture and equipment.

GMP compliance can be achieved when consistent GMP-grade materials from well-characterized sources are implemented and utilized.

Table 1: Cleanroom Classes as defined in GMP

FED209 Approximate Cleanroom Equivalent Class	GMP Approximate Equivalent Class*	ISO Designation	≥0.1 µm	≥0.2 particles/m^3	≥0.3 particles/m^3	≥0.5 particles/m^3
100	Grade A/B	5	100,000	23,700	10,200	3,520
1000		5.5	316,000	74,800	32,200	11,100
1000		6	1,000,000	237,000	102,000	35,200
10,000		6.5	3,160,000	748,000	322,000	111,100
10,000	Grade C	7				352,000
100,000		7.5				1,110,000
100,000	Grade D	8				3,250,000

ISO decimal classes, including ISO Class 5.5, are designated at intermediate thresholds according to ISO 14644-1, Annex E, which states, "specification of intermediate decimal cleanliness classes and particle size thresholds."

* Equivalent GMP Grades as defined by the World Health Organization (WHO).

Table 2: Drug Type and Route of Administration of the Top Drugs of 2019

		Humira	Keytruda	Revlimid	Imbruvica	Opdivo	Eliquis	Eylea	Enbrel	Avastin	Rituxan
Biologic	Intravenous		●			●				●	●
	Subcutaneous	●							●		●
	Intravitreal							●			
Small Molecule	Oral			●	●		●				

Cleanroom Classified Equipment

It is critical to maintain proper attire/gowning, furniture and equipment to minimize the risk of introducing particles and contaminants to cleanroom environments. Equipment not qualified for cleanroom settings can present a variety of hurdles in proper cleanroom maintenance. For example, uncertified tools and equipment with motors and other moving parts can introduce a reservoir of particles into the environment. Some essential tools and equipment are also supplied with engines and other devices that have a multitude of potential particles to shed.

In accordance with ISO 14644-1 and concerning airborne particle cleanliness, "Part 14: Assessment of suitability for use of equipment by airborne particle concentration" was developed to assess the suitability of equipment for use in cleanrooms. This section of ISO 14644-1 outlines the methods used to determine the total particle emission of equipment and provides data that may be used to determine the particle load on a cleanroom. For all equipment classified by an internationally recognized safety certification company, testing must adhere to the methodology of particle emission defined by part 14 of ISO 14644-1. In addition, before any testing can occur, the certification company must ensure all equipment is compliant with the design principles of a cleanroom as defined by part 14 of ISO 14644-1. As described by ISO, these principles ensure that equipment is manufactured with appropriate materials and surface finishes, avoidance of static air zones, design principles of cleanability and considerations for maintenance.

PHCbi Products with a Cleanroom Level of Class 5.0, 5.5 and 6.0 According to ISO 14644-1*

PHCbi main products, cryopreservation equipment and cell culture incubators, has been used in many cleanrooms and cell culture facilities (CASE STUDY).

* The below products manufactured accordingly, has been confirmed to have been evaluated under ISO 14644-14 to be classified into the cleanroom level of each class.

Model Number	Date Manufactured
MCO-80IC-PE	August, 2019
MCO-170AICUVH-PE	October, 2019
MCO-230AICUV-PE	October, 2019
MDF0DU702VX-PE	September, 2019

While maintaining laboratory quality standards has always been critical, the increased focus on strictly controlled environments will continue to expand with stem cell research and regenerative medicine demands. Therefore, as cleanroom classification for GMP manufacturing grows in importance, it is essential to carefully evaluate core laboratory equipment and its role in facilitating cleanroom status.

MCO-170AICUVH-PE MCO-230AICUVH-PE MCO-80IC-PE MDF-DU702VX-PE

Testing Methodology

Each unit has received the appropriate classification after undergoing the following testing methodology:

1. Testing was performed in a dynamic environmental chamber with defined air, temperature, and humidity conditions.
2. The cleanliness of the chamber is calculated according to ISO 14644-14:2016.
3. Device is loaded in the chamber and equilibrated for 24 hours before testing.
4. Then, particle count concentrations were monitored for 120 min. Particle concentrations (number of particles/m³) were measured for particles in size ranges of ⩾ 0.1 µm, ⩾ 0.2 µm, ⩾ 0.3 µm and ⩾ 0.5 µm in diameter.
5. The mean particle concentrations, upper confidence limit in the empty chamber and corresponding ISO Class ratings are calculated and defined according to ISO 14644-1:2015.

PHC Europe offers a full product line that represents more than 50 years of innovation and successful application throughout the life science community. If you are developing GMP manufacturing, we can help.

Contact your local PHCbi brand representative at www.phchd.com/eu/biomedical

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