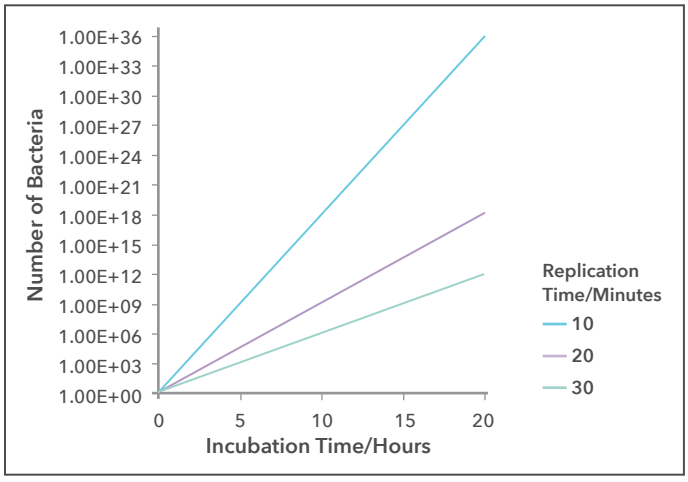
Manufacturing Sterile Products

Introduction

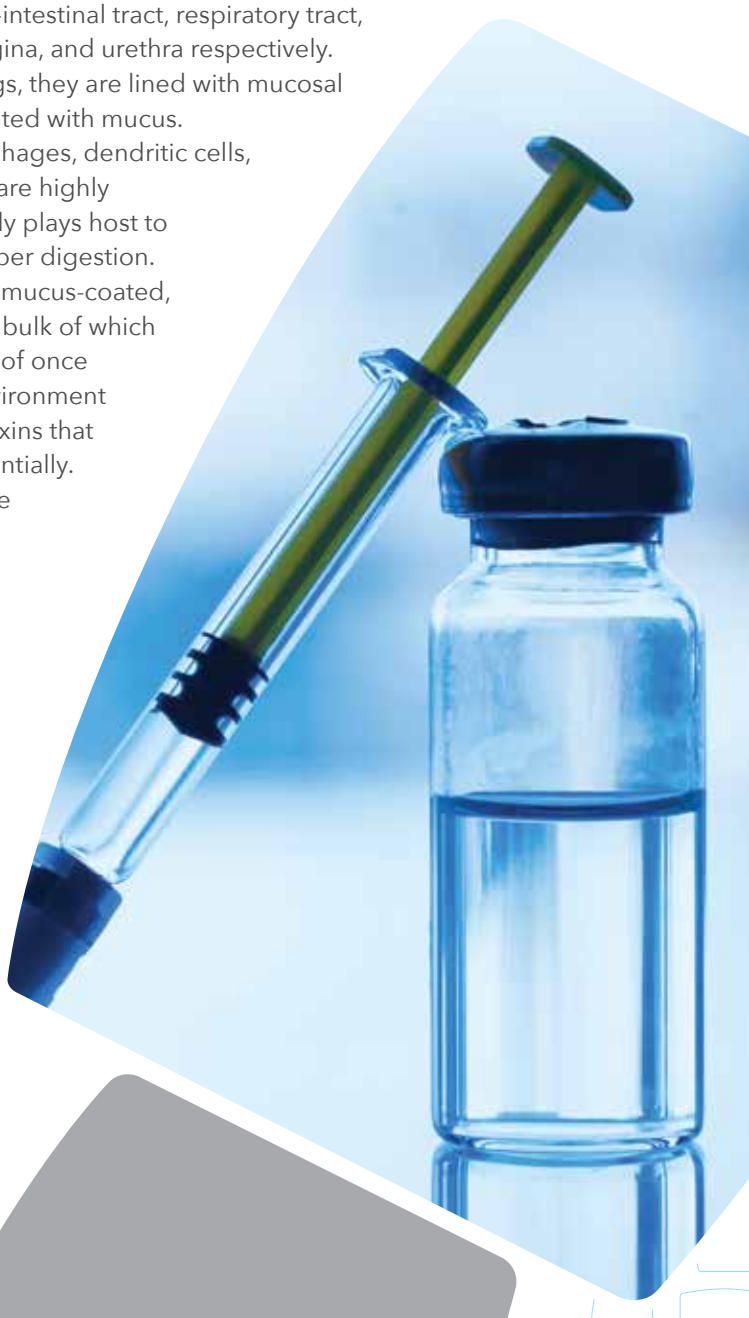
Microorganisms include spore-forming and non-spore forming bacteria, viruses,fungi, and protozoa. There are an estimated five million trillion trillion (5,000,000,000,000,000,000,000,000,000,000) bacteria on Earth[1], more than the number of stars in the entire universe. 99% of the world's bacteria are found in soils, the oceans (to the very bottom), and the atmosphere (to 40 km up). The remaining 1% exist within host animals, often in a symbiotic relationship, and are very well contained within certain organs.

Skin is designed to keep microorganisms out of the sterile innards of the body, and the body has adapted a complex immune system to manage small breaches. A massive invasion can overload the system and be fatal. The necessary openings of the gastro-intestinal tract, respiratory tract, reproductive tracts, and urinary tracts are the mouth, nose, vagina, and urethra respectively. To prevent microbial ingress into the body from these openings, they are lined with mucosal tissue comprising a barrier of tightly bound epithelial cells coated with mucus. Furthermore, killer cells of the immune system such as macrophages, dendritic cells, T-cells, and B-cells, designed to detect and destroy microbes, are highly concentrated in these tissues. The gastro-intestinal tract actually plays host to billions of helpful microorganisms that are required to aid proper digestion. Their containment within the tract is vital and is maintained by mucus-coated, epithelial tissue backed up by cells of the immune system, the bulk of which are found in the GI tract. Bacteria divide rapidly – on the order of once every 10 – 30 minutes. Those bacteria that enter the sterile environment of the body compete with the host for nutrients and release toxins that damage surrounding cells and tissues as they multiply exponentially. Graph 1 shows the number of bacteria generated from a single parent bacterium at different times for different division rates.



Lubrizol Life Science

Graph 1. The number of bacteria generated from a single parent at different times for different division replication times.



LUBRIZOL LIFE SCIENCE

The immune system detects and eliminates pathogenic bacteria but must do so quickly. When the number of bacteria is high, the effects of the immune system working hard to cope are experienced as fever. When the bacterial threat is very large, the results are sepsis that can lead to death. A rupture in the lining of the GI tract releasing the normally helpful bacteria, can cause inflammation of the peritoneum - peritonitis. If not treated quickly with powerful antibiotics this can be fatal, as Harry Houdini discovered when he was unable to escape this final challenge. Since bacteria in the wrong place in the body divide quickly and cause disease, sepsis, and possibly death, pharmaceutical products for introduction to sterile body compartments must themselves be sterile.

Sterile Pharmaceutical Products

Since the GI tract is well developed to tolerate most microorganisms, oral medications (such as tablets, buccal films, sublingual sprays) are generally not made aseptically. Similarly, products for vaginal or rectal administration are not required to be sterile either. Even products for delivery by inhalation to the sensitive tissue of the lungs do not need to be sterile (however they are made under a strictly controlled microbe-free atmosphere). But for drug products that are administered into or which come into contact with the sterile interior of the body, regulators require manufacturers to ensure that products are sterile at the end of the manufacturing process. Such sterile products include parenterals (injectable products), surgical/drug-eluting implants, sutures, and ophthalmic products. Even ophthalmic products for topical administration to the external surface of the cornea are required to be sterile, as the cornea is not well served by immune cells and the eye finds bacterial infection a challenge.

The Sterile Product Manufacturing Environment

Regulators mandate that the manufacture of sterile products must be carried out in a "clean" room specially designed to be free of microbes at all times. The clean room is under positive pressure from an air supply that enters the room via high efficiency particulate air (HEPA) filters designed to trap and remove microbes from the air supply. International Standards Organization (ISO) designates clean rooms according to the number of particles of various sizes found in a given volume of air in the clean room. Table 1 shows these various designations[2]. A clean room for sterile pharmaceutical products must meet at least ISO 5 (class 100).

ISO 14644-1 Standards for Cleanrooms							
Class	Maximum Particles/m3						
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥1 µm	FED STD 209E Equivalent
ISO 1	10	2.37	1.02	0.35	0.083	0.0029	
ISO 2	100	23.7	10.2	3.5	0.83	0.029	
ISO 3	1,000	237	102	35	8.3	0.29	Class 1
ISO 4	10,000	2,370	1,020	352	83	2.9	Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1.0×10^6	237,000	102,000	35,200	8,320	293	Class 1000
ISO 7	1.0×10^7	2.37×10^6	1,020,000	352,000	83,200	2,930	Class 10,000
ISO 8	1.0×10^8	2.37×10^7	1.02×10^7	3,520,000	832,000	29,300	Class 100,000
ISO 9	1.0×10^9	2.37×10^8	1.02×10^8	35,200,000	8,320,000	293,000	Room Air

Lubrizol Life Science

Table 1. Airborne particulate counts for various cleanroom classes, ISO 14644-1

Operators can only enter the clean room via an air lock and only after fully gowning in suits, gloves, and masks that prevent microbes on their skin or clothes from entering the clean room and potentially contaminating the product. The room is frequently cleaned under a strict protocol that dictates the frequency, method, and products to use to clean every surface.

Methods of Product Sterilization

Sterilization is defined by the Centers of Disease Control as "a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores"[3]. There are five main methods of sterilization outlined by the World Health Organization, with recommendations for conditions and validation organisms[4], as follows:

1. Pressurized Steam

Samples are exposed to pressurized steam at elevated temperature in an autoclave. The elevated temperature denatures critical proteins in the microbes. Combinations of suitable sterilizing temperatures and times are shown in Table 3, with recommended conditions being 15 minutes at 121 – 124 °C.

Temperature (°C)	Approximate Corresponding Pressure (Kpa/Atm)	Minimum Exposure Time (Min)
121 -124	200/2.0	15
126 - 129	250/2.5	10
134 - 138	300/3.0	5

Lubrizol Life Science

Table 2. Combinations of heat and time to sterilize by pressurized steam

It is recommended to use spores of Bacillus stearothermophilus to validate a pressurized steam sterilization process. 90% of a population of 106 spores should be destroyed in 1.5 – 2.0 minutes at 121 °C[4].

2. Dry Heat

Samples are exposed to circulating hot air in a suitable oven. The elevated temperature causes oxidation of key molecules in the microbes. Products must be quite stable thermally, as temperatures in this process are higher than for steam sterilization, and times are longer. The process is preferred where steam damages the product or does not adequately penetrate it. Suitable combinations of sterilizing temperatures and times are shown in Table 3.

Temperature (°C)	Minimum Exposure Time (Min)
160	180
170	60
180	30

Lubrizol Life Science

Table 3. Combinations of heat and time to sterilize by dry heat

[3]Centers for Disease Control and Prevention. (2011, January). Healthcare-associated infections (HAIs): Sterilization or disinfection of medical devices. Retrieved June 5, 2012, from http://www.cdc.gov/HAI/prevent/sd_medicalDevices.html.

It is recommended to use spores of Bacillus subtilis to validate a dry heat sterilization process. 90% of a population of 10^6 spores should be destroyed in 5 – 6 minutes at 160 °C[4].

3. Ionizing Radiation

Samples are exposed to high-energy electrons from an accelerator, or gamma rays from the decay of a suitable radioisotope source (usually ^{60}Co). The radiation causes mutations in microbial DNA that interferes with their replication. A sterilizing dose of radiation is typically 25 kilogray (2.5 megarad). The process is very dangerous and is performed in specialized facilities by well-trained personnel.

It is recommended to use spores of Bacillus pumilus to validate a radiation sterilization process, for which 90% of a population of 10^7 – 10^8 spores should be destroyed at 3 kilogray[4]. Other organisms are preferred for higher doses than 25 kilogray.

4. Gas

Considered a last resort where other methods fail, samples are exposed to a biocidal gas, usually ethylene oxide, by skilled workers in a process that can be difficult to control. Temperature and humidity must also be controlled, and there is the risk of explosion as a result of the flammability of the gas used. Packaging of the product must be gas permeable. This process is most suitable to medical devices. Due to the potential for irreproducibility of the process, it is not usually validated – instead the process must be monitored each time using spores of Bacillus subtilis or Bacillus stearothermpohilus. The gas exposure should reduce a population of 10^6 spores by 90%.

5. Filtration

When a drug product is a thermolabile solution, and heat sterilization degrades it, filtration is an option. The solution is passed through a pre-sterilized filter with pore sizes that can be up to 0.45 microns, but are more usually no larger than 0.22 microns. The filter is usually made from plastic (nylon, FTFE, or cellulose-based), sintered glass, or porous ceramic. The integrity of the filter is tested before and after use by determining the pressure at which bubbles of gas form on the membrane after pre-wetting with the liquid. The pressure at which bubbles appear must be within the filter manufacturer's recommendations. The filtered product is introduced to the sterilized primary package via sterilized lines in an aseptic process. The package is sealed immediately after filling to prevent re-contamination.

Guidance on validating a sterilization method is given by FDA[5]. The first four sterilizing methods can be used to sterilize the final packaged product, and therefore termed "terminal" sterilizing approaches. FDA requires that terminal sterilization is used whenever possible to best ensure product sterility. Filtration is not a terminal sterilization process, and is only permitted when the other approaches have been shown to fail – perhaps the product melts, or the drug substance degrades significantly. Manufacturing sterile products using an aseptic process is permitted when it has been demonstrated that terminal sterilization is unsuitable.

Aseptic Processing

Where a manufacturer can demonstrate that terminal sterilization processes are unsuitable for a given product, a validated aseptic process using pre-sterilized raw materials may be permitted. For example, the drug product may be a polymer-based drug-eluting implant which melts at temperatures below the minimum autoclave temperature of 121 °C and also degrades under gamma irradiation. Perhaps an active pharmaceutical ingredient (API) in an injectable drug product is labile, and degrades with heating or exposure to ionization radiation to yield unacceptable impurity levels in the product. Biologics, especially proteins in solution, are delicate molecules, susceptible to coagulation and degradation. The particles in liquid suspensions of a drug product may coagulate at sterilizing temperatures, or the suspension may become unstable under gamma irradiation as stabilizers are degraded. The raw materials may be sterilized by any of the means listed above. Aseptic technique is required throughout the manufacturing process, meaning personnel are trained and adhere to standard operating procedures (SOPs) that minimize the chance of microbial contamination (e.g., wearing proper personal protective equipment; practicing slow, careful movements; and not leaning over exposed product). Most importantly, aseptic processes must be validated for each product and revalidated for any changes – even to container size or batch size. Validation is performed by "media fill", where all the steps of intended manufacturing process that are to be aseptic are carried out in full using bacterial growth media in place of the product. Growth medium is a broth of nutrients that support and encourage bacterial growth. The containers of growth media filled using the proposed manufacturing process are incubated at a temperature that best supports microbial growth. If after a suitable time period, no growth is observed visually, then the process can be considered aseptic. For validation, three consecutive media fills must result in the absence of bacteria in all the incubated samples.

Sterility Testing

According to 21CFR Part 211§167 (a) "For each batch of drug product purporting be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements." The method to test sterility of a batch is described in USP<71>. Depending on unit size and batch size, different volumes from different numbers of units are tested for sterility using growth media. If all test samples are found sterile, then the batch can be released. This does not guarantee sterility of every unit in the batch (which would require the destructive testing of every unit!), but proper statistical sampling methods outlined in USP<71> give sufficient confidence to release the batch.

Summary

Ingress of bacteria into the body's sterile interior can cause irritation, disease, and even death. Therefore, regulators mandate that pharmaceutical products intended for parenteral and ocular administration must be sterile. Sterile products are manufactured by trained personnel in clean rooms meeting at least ISO 5 criteria. Products may be sterilized after manufacture in a terminal sterilization step. If terminal sterilization is not possible, products may be sterilized after manufacture in a terminal sterilization step. If terminal sterilization is not possible, products may also be manufactured from sterilized raw materials, using sterile equipment, through an aseptic process that has been validated by three consecutive successful media fills. Prior to release, each batch of a sterile product must be tested to USP<71> to ensure product sterility. Proper sterile manufacturing and aseptic processing are critical to patient safety and should be performed by organizations with robust training protocols, equipment, and facilities to consistently meet the stringent requirements of these techniques.

References

1. BBC News. *Sci/Tech Planet bacteria*. http://news.bbc.co.uk/2/hi/science/nature/158203.stm. Published 1998.
2. International Organization for Standardization. *Cleanrooms and associated controlled environments - Part 1: Classification of air cleanliness*. https://www.sis.se/api/document/preview/615067/. 1999.
3. Centers for Disease Control and Prevention. *Health-care-associated infections (HAIs): Sterilization or disinfection of medical devices*. 2011 January. Retrieved June 5, 2012. http://www.cdc.gov/HAI/prevent/sd_medicalDevices.html.
4. The International Pharmacopoeia - Ninth Edition. https://apps.who.int/phint/pdf/b/7.5.9.5.8-Methods-of-sterilization.pdf. 2019.
5. Center for Drug Evaluation and Research / Center for Veterinary Medicine. *Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products*. https://www.fda.gov/files/Guidance-for-Industry-for-the-Submission-Documentation-for-Sterilization-Process-Validation-in-Applications-for-Human-and-Veterinary-Drug-Products.pdf. November 1994.

LUBRIZOL LIFE SCIENCE

9911 Brecksville Road
Cleveland, OH 44141-3201 USA

For more information, visit lubrizolcdmo.com
or call us toll free at +1 610-861-4701.

19-216296