Sterilization and sterility assurance

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12.1 Introduction

Many types of pharmaceutical products are required to be sterile. This includes injections, infusions, and pharmaceutical forms for application on eyes and on mucous membranes [1]. These methods of administration are often due to the formulation of the products (e.g., the active ingredient might be inactivated if they were to be ingested). Because of the route of administration, such medicines are required to be sterile. Importantly, if such medicines are not sterile, then this could lead to patient harm or even death.

Manufacturing sterile products not only requires the product solution to be sterile. Sterility must encompass the various components required for the production and development of sterile products. Safeguarding the patient not only extends to manufacturing a sterile product, it needs to include the use of sterile items to administer the drug (such as a sterile syringe and needle) and administering the drug under aseptic conditions, using trained medical or nursing practitioners.

Thus, sterile manufacturing itself is a continuum that stretches from development to manufacturing, to the finished product, to marketing and distribution, and to utilization of drugs and biologicals in hospitals, as well as in patients' homes. There is no generic approach to the manufacturing of sterile products. Each plant or process will differ in relation to the technologies, products, and process steps. The common point is that a product is produced that is sterile and where there is no risk of contamination until the contents of the outer packaging are breached (such as through the injection of a needle through a bung on a product vial).

12.2 Sterility

Sterility can be defined as "the absence of all viable microorganisms." Therefore, something would be deemed sterile only when there is complete the absence of viable microorganisms from it. Sterility is an absolute term. Either something is sterile or it is not. There is no such thing as "slightly sterile" or "almost sterile."

Following on from this, sterilization can be taken to mean the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores. This destruction of bacterial spores means that sterilization is a complete process for the destruction of life, unlike disinfection, which refers to the reduction of a microbial population by destruction or inactivation.

Importantly, this simple definition refers to microorganisms that are "viable" (that is bacteria, fungi, and viruses that are capable of reproducing under the correct conditions). It does not, however, refer to the absence of microbial by-products. By-products

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include toxins that may cause harm, such as endotoxins, exotoxins, or enterotoxins. These can be released by microorganisms as the function or when they die and several toxins are resistant to many types of sterilization (e.g., for endotoxins, a depyrogenation process is required).

Furthermore, the term "sterile" does not extend to other aspects of the formulation, which might cause patient harm, such as the presence of particulates or chemical impurities. Moreover, something that is sterile, such as a liquid in a bottle, is only sterile at a point in time; something that has been rendered sterile can become nonsterile if there is ingress of microorganisms (such as a crack in the bottle leading to microbial ingress). Thus, something that has been rendered sterile is subject to the possibility of becoming nonsterile under certain conditions [2].

Although the definition of sterility—"the absence of all viable microorganisms"—is straightforward, the evidence that something is sterile can only be considered in terms of probability. This is because absolute sterility can only be proved by testing every single item produced (and with technology that will give an undisputable result). However, the act of testing destroys the very item that is required for administration to the patient, so sterility cannot be proven empirically.

Therefore, the concept of what constitutes "sterile" is measured as a probability of sterility for each item to be sterilized [3]. Probability can be considered in relation to components that are sterilized and to products that can be terminally sterilized in relation to a concept called the sterility assurance level (SAL). Importantly, the SAL concept cannot be applied to aseptically filled products. With aseptically filled products, the probability of sterility or nonsterility is the product of environmental controls (from clean air devices and cleanrooms), product filtration, the use of sterilized components, personnel behaviors, and gowning.

12.3 Sterility assurance and the sterility assurance level

The manufacture of sterile products involves the philosophy and application of sterility assurance. Sterility assurance, as a broad term, refers to the philosophy of protecting a sterile product throughout its manufacturing life in relation to controls and practices. It is not synonymous with the SAL, although the reduction of the two concepts is, unfortunately, too common. The term "sterility assurance" is a combination of two words with the following definitions:

- sterility—state of being free from viable microorganisms;
- assurance—a positive declaration intended to give confidence.

Sterility assurance concerns the wider embracement of the aspects of good manufacturing practice (GMP) that are designed to protect the product from contamination at all stages of manufacturing (from in-coming raw materials through to finished products) and, thus, it forms an integral part of the quality assurance system.

A quantitative assessment of the sterility assurance can be provided through the SAL, a term used to describe the probability of a single unit being nonsterile after

a batch has been subjected to the sterilization process (or the probability of a single viable microorganism surviving on or in an item after sterilization) [4]. Importantly, the SAL concept was developed for sterilization processes, and it should be limited to terminal sterilization; thus, it cannot, as a probabilistic concept, be applied to aseptic manufacture (while certain literature attempts to do so, such attempts should be avoided for they are scientifically inaccurate).

A second important point is that the SAL is not exactly a definition of the assurance of "sterility"; rather it is the probability of "nonsterility" [5]. SALs are used to describe the probability that a given sterilization process has *failed* to destroy all of the microorganisms. This is why the term is defined as the probability of a treated item remaining contaminated by one or more viable microorganisms and not, as sometimes misreported, the probability of successful sterilization.

The reason that sterilization is discussed in terms of probability is because it is impossible to prove that all microorganisms have been destroyed. This is because:

(1) microorganisms could be present but undetectable simply because they are not being incubated in their preferred environment;
(2) microorganisms could be present but undetectable because their existence has never been discovered.

SALs can be used to estimate the microbial population that was destroyed by the sterilization process. Each log reduction (10^{-1}) represents a 90% reduction in microbial population. So a process shown to achieve a "6-log reduction" (10^{-6}) will reduce a population from a million microorganisms (10^6) to very close to zero (theoretically). The same logic can apply to containers as to microorganisms. For example, a SAL of 10^{-6} expresses probability of survival, that is, there is one chance in 10^6 that any particular container out of 10^6 containers would theoretically not be sterilized by the process [6].

SAL is demonstrated through validation using innocuous bacterial endospores (biological indicators). The assumption is that the inactivation of such highly resistant microorganisms encompasses all less-resistant organisms, including most pathogens [7].

The concept is also linked to the predictability of microbial death through the use of a defined sterilization process. Microorganisms die when exposed to a sterilization treatment according to a logarithmic relationship, which is based on the proportion of viable microbial cells and the time of exposure to the sterilization process (such as heat) or to the dose (such as radiation).

In assigning a quantitative value a SAL of 10^{-6} takes a lower value but provides a greater assurance of sterility than a SAL of 10^{-3} [8]. Furthermore, the SAL is normally expressed as 10^{-n}. For example, if the probability of a spore surviving was one in one million, the SAL would be 10^{-6}. The reader will note that the SAL is a fraction of one and, therefore, carries a negative exponent (so the six-log reduction is written as 10^{-6} rather than 10^6). However, the reader should be aware that SAL refers to individual items of product and not to a batch of product (Figure 12.1).

This theoretical reduction in microbial population also assumes [9]:

- a single species of microorganism present on or in each product;
- there is a homogenous microbial population;

Figure 12.1 Biological indicators used to determine the sterility assurance level of an autoclave. Photograph: Tim Sandle.

- the population has a mono-disperse distribution on the surfaces to be sterilized, that is, there is no clumping;
- the exclusion of multinucleate spores (e.g., ascospores) or microorganisms.

For many years, a SAL of 10^{-6} has represented the sterilization standard for invasive and implantable devices and medicinal products administered by injection. In practice, many processes use "overkill cycles," which assure an even lower probability that a device will be nonsterile [10].

12.4 Sterility testing

A common means to assess the effectiveness of sterility for medicinal products is, the sterility test. Sterility testing is described in detail in Chapter 6.

Sterility testing is less common for the sterilization of consumables where a terminal sterilization process is used (here the product bioburden is assessed prior to sterilization and compared to validation cycles for the microorganisms found in relation to the population and resistance of the microorganisms to the sterilization process). For medicinal products that can be terminally sterilized, parametric release is often used in lieu of the sterility test (see below). For aseptically filled products, and some terminally sterilized products, the sterility test is a regulatory requirement (as per the US Food and Drug Administration (FDA) and the European Medicines Agency).

Despite the requirement to conduct the sterility test on a representative batch size, it remains that the sterility test is a flawed test on a number of levels. The first relates to the very small sample size tested. Testing any pharmaceuticals and medical devices to a level of statistical significance would require a sample size that would be practically and economically unsustainable. Second, the microbial challenge to the manufacture of pharmaceuticals and medical devices includes microorganisms that, by virtue of their fastidious nature, or physiological prerogative, will not grow on growth medium.

Figure 12.2 Isolator prepared for sterility testing.
Photograph courtesy of Pharmig.

There is a evidence that microorganisms in forms that will not necessarily replicate still retain their ability to cause disease [11].

Validation of the sterility test involves challenging the test with a small number of known microorganisms. Validation of the sterility test is important in order to show that the culture media and the conditions used during the test neutralize any antimicrobial activity that the product possesses, and that microorganisms can be recovered (Figure 12.2).

12.5 Parametric release

Products that can be terminally sterilized can be subject to parametric release without undertaking finished product testing. The European Organization for Quality defines parametric release as: "A system of release that gives the assurance that the product is of the intended quality based on information collected during the manufacturing process and on the compliance with specific GMP requirements related to Parametric Release." Importantly, the organization must demonstrate the capability of the sterilization agent to penetrate to all relevant parts of the product [12].

Parametric release assumes that a robust sterility assurance system is in place, consisting of:

- good product design;
- the company having knowledge and control of the microbiological condition of starting materials and process aids (e.g., gases and lubricants);
- good control of the contamination of the process of manufacture to avoid the ingress of microorganisms and their multiplication in the product. This is usually accomplished by cleaning and sanitation of product contact surfaces, prevention of aerial contamination by handling in cleanrooms or in isolators, use of process control time limits and, if applicable, filtration stages;

- systems for the prevention of mix-up between sterile and nonsterile product streams;
- maintenance of product integrity;
- a robust and consistent sterilization process;
- the totality of the quality system that contains the sterility assurance system (e.g., change control, training, written procedures, release checks, planned preventive maintenance, failure mode analysis, prevention of human error, validation, and calibration).

12.6 Sterile products

There are two main groups of sterile products, related to the way in which they are treated (or not) after being filled into the final container (be that a bag, vial, or syringe). The distinction is between products that can terminally sterilized in their final container and those that cannot due to the effect of the sterilization process upon the product. For example, some protein-based products cannot be subjected to heat. Products that cannot be subjected to terminal sterilization are aseptically filled and rely on the presterilization of the components and bulk product before being aseptically filled within a cleanroom. For these processes, there are different, and higher, levels of risk.

The regulatory bodies, such as the FDA and European regulators, favor terminal sterilization, and, in the development of new sterile dosage forms, the EU regulations demand that a decision tree is followed whereby the new dosage must be proven to be unable to withstand various defined processes of terminal sterilization before it is allowed to be manufactured aseptically. It is important that the organization has selected the appropriate method of sterile manufacturing and is aware of why that method is in place. The preparation of sterile products up to the filling and sterilization of the final product are broadly similar. The two types of sterile product are examined further below.

12.6.1 Terminal sterilization

Both the FDA guidance on aseptic filling (2004) and the European Pharmacopoeia (in Chapter 5.1.1) state that of the methods of sterile manufacture a process in which the product is sterilized in its final container (terminal sterilization) is the preferred method. This is not possible for all types of products and for this filtration through a bacteria-retentive filter and aseptic processing is used.

Terminal sterilization involves filling and sealing product containers under high-quality environmental conditions. This means that non-parenteral products that are to be terminally sterilized may be filled in an EU GMP Grade C/ISO 14644 class 8 area (for detail of cleanroom grades, see Chapter 16). With parenteral products these can be filled under the same conditions if the process or product does not pose a high-risk of microbial contamination. Examples of high-risk situations include slow-filling operations, the use of wide-necked containers or the exposure of filled containers to the environment for more than a few seconds before sealing. In these cases, products are filled in an aseptic area with at least an EU GMP Grade B/ISO 14644 class 7

environments or in an EU GMP Grade A/ISO 14644 class 5 zone with at least a Grade C/ISO class 8 background, prior to terminal sterilization.

Products are filled and sealed in this type of environment to minimize the microbiological content of the in-process product and to help ensure that the subsequent sterilization process is successful. It is accepted that the product, container, and closure will probably have low bioburden, but they are not sterile. The product in its final container is then subjected to a terminal sterilization process such as heat or irradiation. As terminally sterilized drug product, each product unit undergoes a single sterilization process in a sealed container. The assumption is that the bioburden within the product can be eliminated by the sterilization process selected [13].

Product formulation is undertaken at an EU GMP Grade C/ISO 14644 class 8 or an EU GMP Grade D/ISO 14644 class 9 environment. For some higher risk products a pre-filtration through a bacteria-retentive filter may be advisable in cases, particularly where there is a high bioburden. It is up to the pharmaceutical organization to define the level of risk and to justify this to an inspector.

12.6.2 Aseptic filling

Aseptic manufacturing is used in cases where the drug substance is instable when subjected to heat (thus, sterilization in the final container closure system is not possible) or where heat would cause packaging degradation. Aseptic filling is arguably the most difficult type of sterile operations. This is because the end product cannot be terminally sterilized and, therefore, there are far greater contamination risks during formulation and filling. With aseptic processing, there is always a degree of uncertainty, particularly because of the risk posed by personnel to the environment in which filling takes place.

In aseptic manufacture, the dosage form and the individual components of the containments system are sterilized separately, and then the whole presentation is brought together by methods that ensure that the existing sterility is not compromised. Sterility is normally achieved through sterile filtration of the bulk using a sterilizing grade filter (with a pore size of 0.2 µm or smaller) in sterile container closure systems and working in a clean area [14]. This is undertaken in an EU GMP Grade C/ISO 14644 class 8 cleanroom environment. The container and closure are also subject to sterilization methods separately. The sterilized bulk product is filled into the containers, stoppered and sealed under aseptic conditions (under EU GMP Grade A/ISO 14644 class 5 air) within an EU GMP Grade B/ISO 14644 class 7 cleanroom, unless filling is undertaken within a barrier system.

To assist with aseptic processing, engineering and manufacturing technology throughout all industries have evolved considerably. In the context of sterile and aseptic manufacture of pharmaceutical and medical devices, blow-fill-seal (BFS), prefilled syringe filling, restricted access barrier systems (RABS), and isolator technologies represent the main developments. Aseptic processes that exclude human intervention (such as robotics or barrier systems) are at a considerably lower risk than operations that consist of filling machines under unidirectional airflow devices where there is a need for periodic human intervention. With isolator systems the background

environment for the cleanroom can be at EU GMP Grade C/ISO 14644 class 8, based on an appropriate risk assessment. There are additional risk considerations for isolators in that the decontamination procedures should be validated to ensure full exposure of all isolator surfaces to the chemical agent. Aseptic filling is the subject of Chapter 14.

12.6.3 Blow-fill-seal technology

BFS technology is a type of aseptic filling but one at a theoretical lower risk compared with conventional filling. BFS is an automated process where containers are formed, filled, and sealed in a continuous operation without human intervention. This is performed in an aseptic enclosed area inside a machine. The technology can be used to manufacture aseptically certain pharmaceutical liquid dosage forms.

BFS operations are undertaken under EU GMP Grade A/ISO 14644 class 5 conditions with the background environment at EU GMP Grade C/ISO 14644 class 8. Where BFS equipment is used for the production of products that are terminally sterilized, the operation can be carried out within an EU GMP Grade D/ISO 14644 class 9 background environments if appropriately risk assessed [15].

12.7 Sterilization

Although there is a wide variety of mechanisms and processes by which a pharmaceutical or medical device might be rendered free from microorganisms (i.e., sterile), they may be grouped into three main categories. These are [16]:

- *Physical removal*: the complete removal of all microorganisms to achieve a physical absence of microorganisms (such as filtration);
- *Physical alteration*: including physical destruction, disintegration of microorganisms. Altering, changing or deforming the physical cellular or biochemical architecture to destroy all physiological functionality;
- *Inactivation*: the permanent disruption of critical biochemical and physiological properties, potential and the microorganisms propensity (whether active or latent) to realize a clinical condition. Thus, ensuring impotency for generating an infection. For complete assurance of inactivation the microorganisms must, therefore, be essentially "killed" with no residual metabolic activity.

Sterilization is not the same as disinfection. Disinfection is a process that is designed to kill actively growing and vegetative microbial microorganisms to a certain level, and it does not, unless the disinfectant is classified as a sterilant, apply to bacterial endospores. Importantly, disinfection is not a substitute for sterilization [17]. Disinfection is the subject of Chapter 15.

From these important concepts, primary methods of sterilization consist of the following four main categories:

- high temperature/pressure sterilization, principally by dry heat or moist heat. Dry heat sterilization technology is less destructive to many materials than steam, which can be corrosive to metal objects and damaging to certain glass surfaces. However, the heating and cooling

times of dry heat sterilizers often are lengthy. Dry heat at 160 °C (holding temperature for 1 h is required to kill the most resistant spores). A hot air oven is one of the most common methods used for dry heat sterilization.

Steam sterilization is performed under high pressure at temperatures that range from 121 to 140 °C, which is lower than temperatures required for dry heat sterilization and the sterilization times are often shorter. Steam (100 °C) is more effective than dry heat at the same temperature as: bacteria are more susceptible to moist heat, steam has more penetrating power, and steam has more sterilizing power as more heat is given up during condensation.

The classic method of sterilization by moist heat is autoclaving. With this method, sterilization is achieved by steam under pressure, to allow for steaming at temperatures higher than 100 °C. The theory runs that the temperature of boiling depends on the surrounding atmospheric pressure. A higher temperature of steaming is obtained by employing a higher pressure. When the autoclave is closed and made air-tight, and water starts boiling, the inside pressures increase and now the water boils above 100 °C. At 15 pounds per square inch (103 kPascal) of pressure, 121 °C temperature is obtained. This is maintained for 15 min for sterilization to kill spores. It works like a pressure cooker.

- Chemical sterilization: such as gassing using ethylene oxide. The active agent of the gas sterilization process can be ethylene oxide or another highly volatile substance. The sterilizing efficiency of a chemical such as ethylene oxide depends on the concentration of the gas, the humidity, the time of exposure, the temperature, and the nature of the load. In particular, it is necessary to ensure that the nature of the packaging is such that the gas exchange can take place.

The microbicidal activity of ethylene oxide gas is the result of alkylation of protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). Alkylation, or the replacement of a hydrogen atom with an alkyl group, within cells prevents normal cellular metabolism and replication.

An alternative gaseous process is hydrogen peroxide. Within the pharmaceutical industry, this gas is commonly used for the biodecontamination of isolators. Hydrogen peroxide inactivates microorganisms primarily by the combined use of the gas and the generation of free radicals (hydroxyl and hydroproxyl free radicals) during the plasma phase of the cycle.

- Filtration. Filtration concerns the rendering of a liquid or gas as sterile by the physical removal of microorganisms. In order to remove bacteria, the membrane pore size (primarily 0.22 μm) must be smaller than the bacteria and uniform throughout. Filtration processes can be complicated, and the validation is dependent upon the type of material being filtered and its physical properties.
- Radiation sterilization: such as gamma radiation and electron beam. These two radiation processes have similarities and differences and are applicable to different situations and are suited for different materials, in relation to speed of processing, degree of penetration and validation requirements.

There are two types of radiation used for sterilization: ionizing radiation and nonionizing radiation. Ionizing radiation is the use of short wavelength, high-intensity radiation to destroy microorganisms. This radiation can come in the form of gamma- or X-rays that react with DNA resulting in a damaged cell. Nonionizing radiation

uses longer wavelength and lower energy. As a result, nonionizing radiation loses the ability to penetrate substances and can only be used for sterilizing surfaces. The most common form of nonionizing radiation is ultraviolet (UV) light.

The process of selecting the appropriate sterilization method is driven by regulatory, economic, and scientific requirements. In considering the scientific and practical aspects, factors to consider include:

(a) whether the product and its packaging fit into an existing sterilization technology. Ideally, a product will tolerate several different types of technology;
(b) the logistics of transporting the product to and from the site of sterilization. This is obviously easier when the sterilization takes place in-house compared with transporting the product to a contract facility;
(c) validation requirements to verify that the sterilization cycle is effective.

All forms of sterilization have negative effects to a wide variety of packaging materials (and sometimes on the item or product itself). These effects can vary from material to material and between the different packaging components. Sterilization can affect polymers, seal strength, label and box adhesion, corrugated and paperboard strength, and material color. The selection of the sterilization method is, therefore, of considerable importance.

The validation of most, but not all, sterilization processes requires a biological control to supplement physical measurement. This is particularly important for sterilization by heat. For this, biological indicators (spore populations) of bacteria of a known resistance are used. With biological indicators, one common way of quantifying microbial death to a sterilization process is the *D* value. The *D* value is the time or dose required to reduce the microbial population by one log (or 90%, e.g., the time or dose required to reduce a population of 1000–100 cells) [18]. Biological indicators are discussed in Chapter 13.

12.8 Factors affecting sterilization effectiveness

There are a number of factors that affect the success or otherwise of a sterilization process. These are outlined below.

12.8.1 Number and location of microorganisms

All other conditions remaining constant, the larger the number of microorganisms then the time, a sterilization process is required to run for, becomes longer, in order to destroy all of microorganisms present. Reducing the number of microorganisms that must be inactivated through meticulous cleaning and disinfection, or by assembling components within classified cleanrooms, increases the margin of safety when the sterilization process is applied.

In terms of the location of microorganisms, research has shown that aggregated or clumped microbial cells are more difficult to inactivate than monodispersed cells. Microorganisms may also be protected from poor penetrating sterilization methods by

the production of thick masses of cells and extracellular materials, or biofilms [19]. It has also been shown that products that have crevices, joints, and channels are more difficult to sterilize than are flat-surface equipment because penetration of the sterilizing agent to all parts of the equipment is more difficult.

12.8.2 Innate resistance of microorganisms

Microorganisms vary greatly in their resistance to sterilization processes. Intrinsic resistance mechanisms in microorganisms vary. For example, spores are generally the most resistant to sterilization processes because the spore coat and cortex act as a barrier. Implicit in all sterilization strategies is the consideration that the most resistant microbial sub-population controls the sterilization time. That is, to destroy the most resistant types of microorganisms (bacterial spores), the user needs to employ exposure times and a concentration or dose needed to achieve complete destruction [20].

12.8.3 Physical and chemical factors

Several physical and chemical factors also influence sterilization processes, especially temperature and relative humidity. For example, relative humidity is the single most important factor influencing the activity of gaseous sterilants, such as ethylene oxide, chlorine dioxide, and formaldehyde [21]. Whereas achieving a certain temperature is critical for the operation of an autoclave.

12.8.4 Organic and inorganic matter

Organic matter, such as serum or blood, can interfere with the antimicrobial activity of sterilization processes by interfering with the chemical reaction between the certain sterilants and the organic matter resulting in less of the active sterilant being available for attacking microorganisms. The effects of inorganic contaminants on the sterilization process can afford protection to microorganisms thereby limiting the potential effectiveness of the sterilization process.

12.8.5 Duration of exposure

Items must be exposed to the sterilization process for an appropriate minimum time. Most sterilization processes have minimum cycle times, established during validation runs.

12.8.6 Storage

All sterile items should be stored in an area and manner whereby the packs or containers will be protected from dust, dirt, moisture, animals, and insects. The shelf life of sterilization depends on the following factors:

- quality of the wrapper or container;
- number of times a package is handled before use;

- number of people who have handled the package;
- whether the package is stored on open or closed shelves;
- condition of storage area (e.g., humidity and cleanliness);
- use of plastic dust covers and method of sealing.

12.9 Good manufacturing practice

Sterile processing is one of the most regulated areas within healthcare. The reason is due to the potential risk to patients, either directly from the developed drug or through an associated reagent or medical device. If the medicine is contaminated with microorganisms, then the patient may become ill or even die [22].

Any manufacturer of a sterile medicinal product or who operates a sterilization process that links into the production of a sterile product will be subject to a regulatory inspection from their national agency and from overseas agencies if the product is intended for distribution into territories that fall under the auspice of a particular agency. Most regulators adopt a risk-based approach to regulations, guidelines, and inspections. Consequently, risk assessment should be firmly built into the pharmaceutical organization's quality system. Risk management is fundamentally about understanding what is most important for the control of product quality and then focusing resources on managing and controlling these things to ensure that risks are reduced and contained. Before risks can be managed, or controlled, they need to be assessed.

Two important points to remember for any risk assessment are that, first, there is no such thing as "zero risk," and therefore, a decision is required as to what is "acceptable risk." Second, risk assessment is not an exact science—different people will have a different perspective on the same hazard [23].

Regulations relating to pharmaceuticals and microbiological aspects are addressed more widely in Chapter 3.

12.10 Risk assessment

When considering any types of sterile manufacturing, the essential risk must never be forgotten: that the objective is to avoid the contamination of the product by microorganisms or microbial by-products (such as endotoxins). It is also important to focus on the most common sources of contamination. These are [24]:

- *Air*: air is not a natural environment for microbial growth (it is too dry and absent of nutrients), but microorganisms such as *Bacillus*, *Clostridium*, *Staphylococcus*, *Penicillin*, and *Aspergillus* can survive. To guard against this, products and sterile components must be protected with filtered air supplied at sufficient volume;
- *Facilities*: inadequately sanitized facilities pose a contamination risk. Furthermore, poorly maintained buildings also present a risk such as potential fungal contamination from damp or inadequate seals. The design of buildings and the disinfection regimes are thus of importance;

- *Water*: the presence of water in cleanrooms should be avoided. Water is both a growth source and a vector for contamination;
- *Incoming materials*: incoming materials, either as raw materials (which will contain a level of bioburden) or as packaged materials, present a contamination risk if they are not properly controlled. Paper and cardboard sources in particular present a potential risk;
- *People*: people are the primary source of contamination within cleanrooms. People generate millions of particles every hour from activities of breathing, talking, and body movements, where particles are shed from hair, skin, and spittle. Many of these particles will be carrying microorganisms.

These factors should be borne in mind when designing different sterilization processes.

12.11 Conclusion

This chapter has provided an introduction to sterility and to sterilization. The chapter has outlined pharmaceutical microbiology in relation to the ways by which microorganisms can survive within processing environments and thereby present a risk to sterilization or to aseptic filling. The chapter has explained further that sterility is an absolute term, but equally one that is difficult to prove, and thus, it can only be understood in terms of risk and probability. For terminally sterilized products and sterilization processes the sterility assurance concept is useful. This concept cannot, however, be applied to aseptic filling, and instead there is a strong reliance upon environmental controls.

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