Validation of Sterilization Procedures

Although there are a wide variety of mechanisms and processes by whicha pharmaceutical or medical device might be rendered free frommicroorganisms (i.e. sterile), primary methods of sterilization consistof the following four main categories:

1. High temperature/pressure sterilization (by dry heat or moist heat);
2. Chemical sterilization (i.e. gassing using ethylene oxide);
3. Filtration;
4. Radiationsterilization (i.e. gamma)

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

1.DRY HEAT STERILAZATION

Dry heat is one of the most commonly used methods to sterilize and/or depyrogenate pharmaceutical components and products. Dry heat sterilization is often used for heat-stable oils, ointments and powders. Most often, depyrogenation of parenteral containers is performed utilizing a dry heat oven. The depyrogenation process is also utilized on certain heat-stable components, glass containers, metal equipment, etc. to render the item and final parenteral product free of pyrogens. The equipment utilized to provide the dry heat medium must be validated to ensure that the system is able to provide sterile and/or depyrogenated components, on a reproducible basis.

Dry heat sterilization is sterilization by thermal (heat) conduction. The dry heat sterilization process is accomplished by conduction, which is where heat is absorbed by the exterior surface of an item and then passed inwards to the next layer. Eventually, the entire item reaches the peak temperature needed to achieve sterilization. The proper time and temperature for dry heat sterilization, at which microbial kill theoretically becomes effective, is 160°C for 2 hours or 170°C for 1 hour. Dry heat destroys microorganisms by causing coagulation of proteins.

Validation of dry heat sterilization:

Installation Qualification (IQ)

Validation of a dry heat sterilization cycle begins with the execution of the Installation Qualification (IQ) protocol on the equipment (oven, tunnel, or cabinet) which will be used to perform the dry heat sterilization. The IQ protocol verifies and documents that the equipment is installed correctly and meets all of the manufacturer and user requirements. During the execution of the IQ protocol, the equipment drawings, calibration status of critical instruments, instrument and valve information, utility information, and standard operating procedures for the equipment are all confirmed.

Performance Qualification (PQ)

During the PQ**, biological indicators** should be placed throughout the chamber. Typically Bacillus atrophaeus biological indicators are used to demonstrate that the dry heat sterilization process can consistently deliver the required microbial inactivation. The biological indicators used must have a population of $\geq 1 \times 10^6$ organisms.

Incubation and enumeration of the biological indicators demonstrates whether or not the required SAL was reached. If the biological indicator population has been completely reduced (no growth of the biological indicators is observed after incubation), this indicates that the necessary SAL has been reached during the sterilization cycle.

Chemical indicators:

Chemical indicators are defined by the Association for the Advancement of Medical Instrumentation (AAMI) as sterilization process monitoring devices designed to respond with a chemical or physical change to one or more of the physical conditions within the sterilizing chamber. CIs are often used to detect sterilizer malfunction/failures resulting from improper loading of the sterilizer, incorrect packaging, deficiencies of the sterilizing agent, or malfunction of the sterilizer itself.

Remember, the pass reading of a CI does not mean that the item or items in the sterilizer load are sterile; it means that the parameter or parameters for sterilization that the CI was designed to measure have been met. The use of CIs is only one portion of an effective quality assurance program. They should be used in conjunction with a biological indicator (spore test), physical

monitors, a sterilizer preventative maintenance program, and accurate record keeping for each sterilization load.

Physical indicators:

Monitoring physical indicators involves observing the gauges or displays on the sterilizer and recording the time, temperature, and pressure associated with each sterilization cycle for each load and comparing them with standard chart record. Some sterilizers have recording devices that print out these parameters. Correct readings do not guaranty sterilization, but incorrect readings can be the first indication of a problem with the sterilization cycle and suggest the load may not be sterile.

BATCH OVEN VALIDATION

1. **Air balance determination**: Air should be balanced so that positive pressure is exerted to the non sterile side when the door is opened

2. **Heat distribution of an empty chamber:** Thermocouples should be situated according to a specific predetermined pattern. Repeatability of temperature attainment and identification of the cold spot can be achieved if the temperature range is ±15℃ at all monitored locations. Heat-distribution studies can also be conducted as a function of variable airflow rates.

3. Heat-penetration studies: These studies should be designed to determine the location of the slowest heating point within a commodity at various locations of a test load in the sterilizer. Thermocouples are placed in the commodities located in the areas likely to present the greatest resistance to reaching the desired temperature. Normally, three replicate cycles are run at Minimum and maximum temperatures. The cold spot must not move during the replicate studies. Other variations in the cycle affecting heat penetration at the cold spot can be studied, and these might include (a) test load variations, (b) temperature set point variations, and (c) variations in the time of exposure.

4. Mechanical repeatability: During all these studies, mechanical repeatability in terms of air velocity, temperature consistency, and reliability and sensitivity of all the oven and instrumental controls must be verified.

2. STEAM STERILIZATION

Definition

''Exposure of microorganisms to saturated steam under pressure achieves their destruction by the irreversible denaturation of enzymes and structural proteins.''

Basic principle

The basic principle of steam sterilization, is to expose each item to direct steam contact at the required temperature and pressure for the specified time.

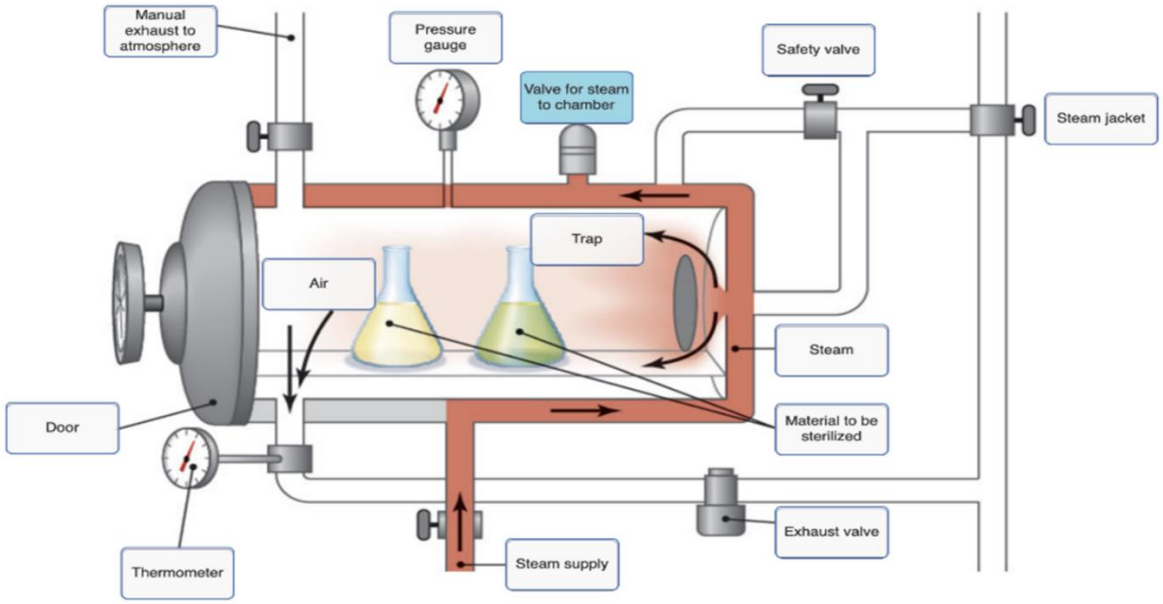
Thus, there are four parameters of steam sterilization: steam, pressure, temperature, and time.
The ideal steam for sterilization is dry saturated steam and entrained water (dryness fraction ≥97%) Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms. Specific temperatures must be obtained to ensure the microbicidal activity.
The two common steam-sterilizing temperatures are 121°C (250°F) and 132°C (270°F). These temperatures (and other high temperatures) must be maintained for a minimal time to kill microorganisms. Recognized minimum exposure periods for sterilization of wrapped healthcare supplies are 30 minutes at 121°C (250°F) in a gravity displacement sterilizer or 4 minutes at 132°C (270°F) in a pre-vacuum sterilizer. At constant temperatures, sterilization times vary depending on the type of item (e.g., metal versus rubber, plastic, items with lumens), whether the item is wrapped or unwrapped, and the sterilizer type.

Mechanism of action:The mechanism by which populations of microorganisms are inactivated at high temperatures in the presence of steam (moisture) and in the absence of air is one where the energy input from the steam inactivates microorganisms by denaturation and coagulation of their intracellular proteins.
In addition, moist heat causes irreversible damage to macromolecules, primarily to cellular structural proteins. It is thought the destruction of cells by lysis may also play a role.

STEAM STERILIZATION DEVICES AUTOCLAVE

The name comes from Greek *auto*-, ultimately meaning self, and Latin *-clavis* meaning key— a self-locking device.

History: The autoclave was invented in 1879 by Charles Chamberland, but the concept of using steam in an enclosed space for the purpose of preventing sickness had already existed since 1679.



Autoclaves are commonly used in healthcare settings to sterilize medical devices. The items to be sterilized are placed inside a pressure vessel, commonly referred to as the chamber. Three factors are critical to ensuring successful steam sterilization in an autoclave: time, temperature and steam quality.

TEMPERATURE AND TIME RELATIONSHIP IN STERILIZATION.

Higher temperatures/Pressure values ensure more rapid killing. Some standard temperature/pressures employed are 115ºC/10 p.s.i., 121ºC/15 p.s.i., and 132ºC/27 p.s.i.

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The thermal resistance of specific microorganisms is characterized by “D”–values and “Z”–values.

D-value is the time in minutes, at a specific temperature, to reduce the surviving microbial population by 1 – log. It is the rate of killing of the microbes D-value is important in validation of sterilization because it is particular for each microorganism and the knowledge of D value at different temperatures is necessary for the calculation of Z-Value.D-value can predict the reduction of microbe population.

Z-value is the temperature required to result in a 1-log reduction in D-value. The standard Z-value for the Bacillus stearothermophillus spores is 10℃

Fo-value: is the number of minutes to kill a specified number of microorganisms with a Z-value of 10° C at a specific temperature of 121.1℃.

QUALIFICATION OF AN AUTOCLAVE FOR STEAM STERILIZATION

Validation is the documented act of proving that any procedure, process, equipment, material, activity, or system actually leads to the expected results. A system must be qualified to operate in a validated process. So the qualification of the system and/or equipment will lead to the Validation of a process, e.g. you qualify an autoclave, whereas you validate a sterilization process.

Validation: The objective of process validation is to prove that the process is effective and can deliver a product (result) that is reproducible and meets the required quality require. The main focus is therefore on the process itself and proof of compliance with the acceptance criteria that is to be defined by the system operator.

Equipment Qualification

Prior to commencing heat distribution, heat penetration and/or biological challenge reduction studies, it is necessary that the equipment be checked and certified as properly installed, equipped and functioning as per its design.

DQ – Design Qualification

The first qualification stage of an autoclave starts as soon as you formulate and document your requirements for the device to be purchased. This means you have already created your User Requirement Specification (URS); i.e. the requirements that are made of a device and which must be fulfilled. For many manufacturers, autoclaves can be equipped with options that

influence process accuracy or speed, for example. If certain options depending on the processes to be carried out are not taken into account in the URS, and if they are not upgraded according to the device, this can't ensure successful qualification and validation.

After a successful DQ, the device is manufactured by the manufacturer according to the agreed specifications. At the same time preparing the documents required for the subsequent steps IQ, OQ, and PQ (validation plan) can be started. These documents form the basis for implementing the IQ, OQ, and PQ and specify the inspections and tests to be carried out as well as their parameters and acceptance criteria.

IQ – Installation Qualification

After the design qualification has been successfully completed and the device has been successfully delivered, the next qualification stage of the autoclave can begin on site – the installation qualification (IQ). The suitability of the installation site, the physical characteristics of the supplied autoclave, as well as the completeness of the documentation belonging to the device are now checked and documented in the qualification documents. Documentation generally includes the operating instructions, maintenance, cleaning, and calibration instructions as well as material certificates and data sheets for individual installed components.

OQ – Operational Qualification

Operational Qualification (OQ)- process of obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures .

The OQ process verifies that the autoclave meets the desired and intended performance standards of the lab. OQ testing examines the autoclave's ability to run the sterilization process correctly and repeatedly while also responding appropriately to error conditions. OQ testing typically includes Empty chamber temperature mapping, Alarm conditions etc.

PQ- Performance Qualification

A process which obtains and documents evidence that the autoclave, as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and thereby meets its specification

PQ demonstrates that the autoclave not only runs the required cycles, but provides the required result. The PQ process involves testing the loads that must be successfully sterilized in the autoclave. The prerequisites for PQ are successful IQ and OQ

1. Bowie- Dick test:

Objective: To ensure that the vacuum pulses applied before the sterilization hold period are sufficient to remove the entrapped air so as to facilitate the event and rapid steam penetration into all parts of load and maintaining this condition during sterilization holding time.

Procedure: Place the Bowie Dick test paper on the bottom shelf of the sterilizer just above drain point (100mm over the drain). Air removal study shall be performed in empty chamber by placing the Bowie Dick test paper. It consist of standard paper pack and indicator sheet. Sterilizer vacuum performance is acceptable if the sheet inside the test pack shows a uniform color change. Entrapped air will cause a spot to appear on the test sheet, due to the inability of the steam to reach the chemical indicator.

Acceptance criteria: The Bowie dick indicator should show uniform color change (Yellow to Brown / black) after the cycle. No change or no uniform change or air entrapment (bubbles) spot on the test sheet indicates inadequate air removal from the sterilization base chamber.

2. Vacuum Leak Test:

Objective:

To verify the leakage in sterilization chamber during vacuum hold time when the sterilizing chamber is empty.

Principle: These tests are designed to show that the sterilizer chamber does not leak in empty chamber.

Leakage of air into the chamber is not acceptable for two reasons:

1. The presence of air inhibits penetration of the load by the sterilant (Steam) and prevents sterilization.
2. Air leaking into the chamber during the sterilization cause a risk of contamination of the load.
Acceptance Criteria: The rate of pressure change shall not exceed 0.13 kPa/min over a 16 minute pressure hold phase

3. Heat Distribution Study (Empty Chamber/Loaded Chamber)

Objective: To verify the temperature uniformity throughout the chamber and to locate the cold spot in

Procedure: Insert 16 no of temperature sensors (Thermocouples) inside the chamber through the validation port of sterilizer. Connect the temperature sensors to the data logger, which can scan and print the actual temperature and pressure at different locations.

Acceptance criteria: Temperature distribution within the chamber must be between 121℃ to 123℃ at all location during the sterilization period (dwell time)

4. Heat Penetration Study/ Loaded Chamber Microbiological Challenges Studies

In order to verify that the sterilizing temperature has been reached in each load subjected to moist heat sterilization, it is necessary to conduct heat penetration studies. These studies are conducted to ensure that the coolest unit within a pre-defined loading pattern.

Heat penetration studies should be performed according to detailed written procedures using temperature sensing devices which have been calibrated before and after each validation run which are capable of simultaneous data generation within pre-established time intervals in order to permit determination of the slowest and fastest heating units in the chamber.

5.BIological Challenge Reduction Studies

The microorganisms most frequently used to challenge moist heat sterilization cycles are *Bacillus stearothermophilus*. Spores of this microorganism in the form of ampoules are used as Biological Indicators. Each BI will contain at least 10^6 viable spores of microorganisms. After the sterilization cycle is complete, the spore ampoules are recovered & subjected to microbiological test procedures. Spores are aseptically immersed in a suitable growth medium

(soyabean digest medium is typical) & incubated for up to 7 days. Incubation temperature for B. *stearothermophilus* is 50-55 degree celsius. The acceptance criterion should be for no survivors (i.e. each BI must exhibit no growth at the end of incubation).

The destruction of the BIs with 10^6 spores and a D-value 1.5 minutes, demonstrates that the thermal lethality delivered is not less than an F_0 of 12 minutes. By adopting the standard pharmacopoeial cycle 121℃ for 15 minutes, the desired level of microbial destruction is easily achieved with a degree of overkill (in this case by 3 minutes). Here thus, the Sterility Assurance Level (SAL) of 10^{-6} is achieved

REQUALIFICATION

Once validated, the requalification of steam sterilization is usually undertaken at annual intervals or where significant changes to established loads are proposed. The purpose of requalification is to determine if any unforeseen change has arisen that might affect the sterility assurance provided to the items being sterilised. It is important for requalification that the numbers, resistances and substrates for the BIs closely similar to those used in the initial validation. All failures must be investigated and the steriliser should not be used until the root cause has been determined and successful sterilization cycles.

3. STERILZATION BY FILTERATION

Filtration is a means of sterilizing fluids (liquids or gases) through the removal, rather than the destruction or inactivation, of microorganisms. Liquids that would be damaged by heat (i.e. those containing proteins such as large molecule drug products) irradiation or chemical sterilization can only be sterilized by filtration. Thus the sterilization of liquids is a key step for aseptic manufacturing, as the means of passing the bulk product to the point where it will be dispensed into bottles. Due to the small pore size of the filters, the term ‘microfiltration’ is used. Sterilization, in relation to filters, generally refers to the removal of bacteria and fungi. In processing of certain biologics, viruses must be removed or inactivated using nano-filtration. This requires a Nano filter with smaller pore size of 20–50 nm.

The ideal characteristic of a sterilising grade filter is in selecting a filter that is compatible with the process. The filter must be non- toxic, able to be tested using the integrity test, and

sterilisable (or provided pre- sterilised). Furthermore, the selected filter must not adsorb formula components or add extractables to the process; and it must, most importantly, remove the bioburden associated with the product. Sterile filtration of liquids and gases in pharmaceutical manufacture is almost always performed using membrane filters. These are thin uniform porous sheets, which act as sieves that trap particles larger in size than the pores in the membranes.

Sterilizing grade filters:

The objective of filtration is for a fluid (gas or liquid) to be passed through the filter and for the filter to capture and retain particles. The process of sterile filtration is one of bacterial retention. The US Food and Drug Administration (FDA) define a sterilising filter as: 'one which when challenged with the microorganism ***Brevundimonas diminuta*** at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent'. Filters are classified in different ways, one of which is by their removal rating, which relates to the size of microorganisms and particles that can be theoretically removed by the filter, rather than the actual size or shape of the filter.

Validation of sterilising grade filters

The purpose of sterile filtration validation is to prove that a particular filtration process generates a sterile filtrate. Validation of sterilising grade filters can be divided between tests of the bacteria- retentive properties and of physico-chemical interactions between the filter and the gas or liquid being sterilised. Filters must be qualified by the user to demonstrate that their performance in processing will meet process requirements. These tests are as described below.

1. ***Physical and chemical compatibility***

The filter system must be qualified to ensure that all product-contact surfaces of the filter and its constituent parts (membrane, support layers, core, cage, and end caps), o-rings, piping, hoses, seals, pumps, gaskets, and any other components of the sterilizing filtration system can withstand the hydraulic, thermal, and chemical challenges of the sterilization and production processes. None of these should extract into the filtered pharmaceutical product in any significant amount

2. ***Binding and adsorption filter characteristics***

These characteristics are measured during the qualification phase from both pre- and post-filtrate testing. It is important that the filter does not remove active ingredients, excipients, carriers, diluents, proteins, preservatives, or any other formulation component, otherwise the properties of the product will be affected and the yield will be reduced.

3. Bacterial retentive efficiency

The validation of bacterial retention requires, according to CGMP standards, the complete removal of a minimum challenge level of 10^7colony forming units (cfu) of *Brevundimonas diminuta*(ATCC 19146) per square centimetre of membrane surface area. This ensures that a sufficient challenge is given to the membrane, so that every pore is challenged and given the same opportunity to allow passage of the test microorganism.

The reason why *Brevundimonas diminuta* is used for this challenge is because the microorganism can be consistently cultured under controlled conditions to produce very small, monodispersed cells with a narrow size distribution.

Due to the specialized nature of the test, the assessment is normally performed only by the filter manufacturers, who then provide limits for secondary physical tests (i.e. bubble point, pressure decay, forward flow, and so forth). These can subsequently be applied to verify the pore size rating and integrity of the membrane filters. Should the filter fail to retain microorganisms, an investigation is required. The retention of microorganisms by the filter is a combination of different factors. These include the filter polymer, the filter structure, the properties of the aqueous product including pH, viscosity, osmolarity and ionic strength, and the process conditions, including temperature, differential pressure and flow rate. The investigation may lead to process modifications or the selection of an alternative filter.

4. Integrity testing

Although the integrity test is an important part of pre- and post- use assessment of the selected filter, such testing should also feature during the initial validation, in order to determine if the filter can be satisfactorily tested prior to implementation.

5. Toxicity and extractables

To demonstrate that the filter must be non- toxic, it should be examined according to specified tests. The *European Pharmacopeia* does not specify such tests; however, there are applicable chapters in the *USP*, which should be followed. These are: chapter 'Biological Reactivity Tests,' *in vitro*; and chapter 'Biological Reactivity Tests,' *invivo*. In addition, filters must be free of bacterial endotoxins and, depending upon the process requirements, free from beta glucan (these are tests normally undertaken by the manufacturer and certified).

The validation of membrane filters must also address the possibility of products leaching harmful 'extractables' out of the plastics. This type of validation is formulation- specific. In addition to the potential adverse effect of extractables on the filtered product, the presence of extractables may be related to degradation of the filter, which will affect its ability to perform as intended. Extractables are chemical entities, both organic and inorganic, that will extract from the filter into the product under controlled conditions. Consideration must also be given to leachables. Leachables are chemical entities, both organic and inorganic, that could migrate into the drug product following contact with the filter. With filters used for the sterile filtration of gases, the FDA requirement for bacteria- retention is for filter suppliers to undertake the same test as with the microorganism suspended in water. It can be reasoned that hydrophobic filters have all the bacteria- retentive mechanisms of hydrophilic filters plus some more mechanisms, therefore if they meet the standard when wet they will more than fit the bill when dry.

4. GASEOUS STERILIZATION

There are different types of gaseous sterilization. Sterilizing gases include formaldehyde, ethylene oxide (EO), and propylene oxide, ozone, per acetic acid, vapor hydrogen peroxide and chlorine dioxide [1]. Most common to sterile manufacturing is EO, which is used to sterilize many plastics, and vapor hydrogen peroxide, which is used to decontaminate barrier systems (i.e. isolators). Gaseous sterilants are effective surface sterilizing agents, in that they will sterilize the outside of a device, or the primary packaging in which the device is held. The key parameters affecting the effectivity of gas sterilization are active concentration, temperature, duration of exposure and relative humidity. Aside from steam (moist heat) sterilization, EO sterilization is the most widely used method of sterilization in the medical device and biopharmaceutical sectors. EO is commonly selected for objects sensitive to temperatures greater than 60 ℃ and/or to radiation. Due to its ubiquity, this chapter focuses foremost upon EO; with reference made to

chlorine dioxide and ozone. In the past, other gaseous agents have been used, such as glutaraldehyde and formaldehyde. Due to the toxicological concerns associated with such agents, they are no longer used.

Sterilization Cycle:

The sterilization cycle consists of:

Preconditioning phase, which is the treatment of the product prior to the sterilization cycle to attain a predetermined temperature and relative humidity throughout the load; the actual sterilization run, which is the exposure to EO in a sealed chamber. The key step here is the gas time, which is the time elapsed from the start of EO injection into the sterilization chamber until the desired gas concentration is attained;

The removal of EO; a post- sterilization aeration period.

The objective of the aeration period is to remove toxic residues, such as EO residues and by-products, such as ethylene glycol (formed out of EO and ambient humidity) and ethylene chlorohydrine (formed out of EO and materials containing chlorine, i.e. PVC, a common component of many plastics). For aeration, there are different aeration technologies available, such as pulsed vacuum and heat addition, steam addition and removal, as well as combinations of different gases and pressure set points. Novel developments include microwave desorption. After the aeration stage, the cycle is complete and the load may be removed from the chamber.

Validation of ethylene oxide sterilization cycles

When using gaseous systems, the initial validation is of great importance, as it provides assurance against the possibility of non- sterility. When preparing validation reports, key parameters should be established including temperature, relative humidity and gas concentration.

There are three microbiological approaches for process validation:

- overkill method;
- combined biologic indicator
- Bioburden method.

Of these, the overkill approach is the most robust and involves the use of biological indicators (*Bacillus atrophaeus*) with a defined spore population and D-value. Validation begins with assessing the material in the sterilizer and measuring physical variables. This is to establish the worst- case location or locations, and temperature fluctuations are commonly taken for this measure. Once the worst- case location(s) is identified for a given sterilization cycle, validation

studies are conducted with the goal of inactivating a known concentration of the biological indicator microorganisms in the worst- case location. Cycle According to this method, at least a 6-log reduction in population of microorganisms must be obtained for each biological indicator in the half cycle. Using the same process parameters, except exposure time, the full sterilization cycle theoretically achieves at least a 12-log reduction by doubling the half cycle time.

The validation of gaseous sterilization procedures includes an assessment of:

- Product bioburden, an assessment of 10 items from a minimum of 3 production lots;
- Manufacturing area environment, to ensure that the clean environment in which the product is manufactured does not pose a contamination risk to the product;
- Determination of time and humidity in the preconditioning area;
- Determination of temperature, pressure, time and humidity in the chamber;
- ventilation of load after sterilization;
- Loading patterns;
- Biological indicator survival;
- Vendor certification, if the gaseous sterilization treatment is carried out by an external contractor.

RADIATION STERILIZATION

Radiation Sterilization Validation determines the appropriate radiation sterilization dose for a product that requires a sterile label claim. It incorporates bioburden testing, a bioburden recovery efficiency test, a sublethal radiation dose (verification dose), a test of sterility, and bacteriostasis/fungistasis testing.

Radiation validations consist of three main phases:

1. Bioburden test: This test determines the quantity of viable microorganisms on or in the product. This test is performed prior to any sterilization but after all other manufacturing steps, including packaging.

2. Application of verification dose: The bioburden results are taken to a table from the standard to determine the proper verification dose. The verification dose is then applied to the required number of products. Samples should be double bagged prior to dosing. This provides an extra barrier to take into the cleanroom and can reduce the potential for false positives.

3. Sterility test: The irradiated products undergo a test of sterility. The number of samples required for sterility testing depends on the test method used. A VDmax method requires 10 non-sterile samples from one production lot. Samples are irradiated at the calculated verification dose followed by the sterility test. For a single lot validation, 10 samples from the lot being validated will be irradiated and tested for sterility. This will give an SAL-1, which means that if one sample is positive out of 10 samples tested, sterility test passes.