1 Purpose

To provide guidelines for the validation of sterilization processes used in the manufacturing activities for drug products or active Pharmaceutical ingredients (API) and also to outline recommendations on how to achieve compliance.

2 Scope and Applicability

This Guideline is applicable to all manufacturing Operations, sites, functions and departments undertaking work, or providing support services, required to meet Good Manufacturing Practice (GMP) or, in the absence of a GMP standard, International Organization for Standardization (ISO) standards.

This Guideline is applicable for sterilization processes used to produce sterile drug products, components, equipment and other ancillary items required to be sterile for use in the drug manufacturing process.

3 Definitions

3.1 D value

The time in minutes at a specific temperature required to reduce a surviving microbial population by 90%, i.e. a one-logarithm reduction.

3.2 F0

The time required at any given temperature between 100°C -140°C that is equivalent to the sterilization effect of steam at 121.1°C (250°F). Assumes a Z value of 10°C.

3.3 Z value

The number of degrees Celsius required to change the D value by a factor of ten.

3.4 Sterile

State being free from viable microorganisms. In practice no such absolute regarding the absence of microorganisms can be proven.

3.5 Sterilization

Validated process used to render a product free of all forms of viable microorganisms. In a sterilization process, the nature of microbial death is described by an exponential function. Therefore, the presence of microorganisms on any individual item/container can be expressed in terms of probability. While the probability may be reduced to a very low number, it can never be reduced to zero.

3.6 Overkill cycle

A sterilization cycle that provides a 12-log reduction of a resistant Biological Indicator (BI) with a known D value of not less than 1 minute. A typical cycle would provide a minimum

4 Responsibilities

All sterile manufacturing sites or its contractors are responsible for ensuring that sterilization processes used to produce items are properly validated.

5 Guideline

Validation of processes used to sterilize drug products and equipment are the most critical validation activities undertaken. Common elements in the validation of any sterilization process include:

- Sterilization Cycle Development
- Biological and Physical Measurement Controls
- Empty Chamber Studies
- Loaded Chamber Studies
- Routine Use/Ongoing Monitoring
- Validation Maintenance/Change Control/Revalidation

The sterilization method chosen depends on the application. The following methods are typically available:

Method	Typical application
Steam sterilization	For the sterilization of fluids in ampoules, vials etc, or the sterilization of processing equipment, reactors, preparation tanks, solution delivery piping, etc. In general, sterilization through the application of saturated steam under pressure is the preferred method of sterilization. The principles apply to SIP processes as well.
Sterilization by filtration	Used for those products that cannot be sterilized due to the heat sensitivity of the product or where heat labile packaging is chosen since it provides a distinct patient benefit. Not the preferred sterilization method.
Dry heat sterilization and Depyrogenation	Used to sterilize/depyrogenate containers (ampoules, vials, etc.), pharmaceutical raw materials and processing equipment. The use of dry heat has little application for the sterilization of pharmaceutical drug products.
Radiation sterilization	To sterilize packaging equipment, consumables, garments etc. that are difficult to sterilize using steam or other methods. Radiation sterilization is mostly used for medical devices.

5.1 Steam Sterilization

Steam Sterilization is the most common type of sterilization employed in the pharmaceutical manufacturing environment. The principles of steam sterilization are applicable to processes conducted within autoclaves as well as sterilization-in-place (SIP) processes.

For the sterilization of fluids in e.g. vials and ampoules, a fluids load autoclave cycle is used. The steam (or superheated water) is used as a heat transfer medium to heat the contents of the vials/ampoules. The moisture required for sterilization is derived from the contents in the vial/ampoule.

degradation of the product does not occur.

Product Specific Method

The Product Specific Method (PSM) applies to sterilization cycles where an overkill approach is not possible because the product cannot withstand the temperatures and/or exposure times required for an overkill cycle. The PSM is more applicable to the validation of heat sensitive fluids, for example, where the sterilization cycle is developed to adequately destroy the microbial load and yet not result in product degradation.

The PSM relies on determining the population and heat resistance of the environmental, product, or items-to-be-sterilized bioburden along with ongoing monitoring and control over the bioburden during routine operation. A PSM cycle would deliver less lethality than an overkill cycle, but would still deliver a Sterility Assurance Level of at least 10-6 (for the most heat resistant bioburden in the product).

In the PSM, once the population of the overall bioburden and the heat resistance (generally only for spore-forming environmental or product/item isolates) has been determined, these values (plus additional safety margins based on professional judgment, the extent of the bioburden data, and the degree of product bioburden testing that will be conducted on an ongoing basis) are used to determine the lethality necessary to achieve a minimum SAL of 10-6.The safety margin selected inversely correlates to the frequency and magnitude of ongoing tests conducted for bioburden population and resistance. For example, if the observed worst-case product spore bioburden resistance is 0.3 minutes and, for lethality determination, a D-value of 0.4 minutes is selected, then extensive ongoing bioburden resistance testing would be necessary. However, if a D-value of 1.0 minute were selected for lethality determination, then minimal ongoing bioburden resistance testing would be necessary.Ongoing monitoring of bioburden population must be conducted.

Once the bioburden data has been used to determine the lethality required for the PSM derived cycle, the attributes (population, resistance) of the BI challenge system can be determined. The semi-logarithmic survivor curve equation is used for determination of both the required cycle lethality and the attributes of the BI challenge.

When the resulting log reduction for the BI challenge system is compared to the log reduction of the typical bioburden organism (whose D value is substantially less) the log reduction delivered for the actual bioburden is significantly higher than the demonstrated log reduction using the BI challenge system.

The F-value for steam sterilization (F0) is a measurement tool used to demonstrate accumulated lethality and must be used in both validation and routine monitoring of cycles to demonstrate that acceptance criteria for F0 has been met.

For terminally sterilized drug products validated using the PSM approach, reductions in cycle parameter set points during the validation are less common.

Terminally sterilized drug product cycles generally include an upper limit for the

allowed to dry on the surface of the item and then the item is positioned in the sterilizer load. Following the validation cycle, the item is recovered aseptically and tested in a manner similar to that used for the spore strip or coupon. Spore suspensions may also used during the validation of terminal sterilization of drug products or other liquids by direct inoculation into the liquid filled container. The container is placed in the load, subjected to the validation cycle, recovered, and its contents subject to filtration and incubation similar to a sterility test. Spore suspensions can be used to inoculate fluid pathway surfaces such as the interface between a vial stopper and the stopper-seating surface of the vial. Use of spore suspensions to inoculate surfaces or liquids requires that the population and D-value be determined for the conditions and substrates involved. The BI manufacturerôs labeled D-value should only be considered a very rough estimate of performance. The BI manufacturer may offer a service to determine the D value of the spores in the product to be sterilized.

5.2 Sterilize-In-Place

Sterilize-In-Place (SIP) processes follow the general principles covered for conventional steam sterilization validation with some unique considerations. A true saturated steam environment is not always obtained in a SIP system and air and condensate removal present design and operational challenges. The overkill cycle design is typically implemented for SIP processes.

Cycle development studies must take into consideration the necessary controls to ensure that air and condensate are removed from the system and that the steam quality characteristics, particularly non-condensable gasses are controlled.

Controls and instrumentation to monitor and/or control the pressure differential across filters, steam flow direction across filters, monitoring of temperature at various locations and verification of proper steam trap operation, the cooling process and proper conditions in the system post-sterilization to maintain sterility are also parameters to be addressed.

Purge times, steam valve pressure settings, temperature ranges, time for heat-up, exposure time, exposure temperature and cooling/drying times are typical cycle parameters to define and control.

The validation includes both the physical and biological assessments already covered for steam sterilization.

5.3 Filtration Sterilization

The use of microbial retentive filtration to achieve sterilization of product solutions or process gasses must be validated. The current widely accepted definition of a sterilizing grade filter is one that is designated as nominally 0.22 µm porosity or smaller.

The EMEA requires that product solutions, before filtration, contain not more than 10 colony-forming units (CFU) per 100 mL. If the pre- filtration bioburden limit is greater than 10 CFU per 100mL, then a bioburden reduction filtration is required to reduce the bioburden down to not more than 10 CFU per mL. The product solution is then filtered using a sterilizing grade filter.

5.4 Dry Heat Sterilization and Depyrogenation

Dry heat sterilization processes are typically used for equipment parts while dry heat-based depyrogenation processes are used for glass containers for drug products. Dry heat sterilization and depyrogenation can be performed in ôbatchô dry heat ovens or continuous sterilizing tunnels. Rubber closures are also subject to a validated depyrogenation process using a washing/rinsing process - See section 5.3.4 below). There is limited use for dry heat in sterilizing drug products, though raw materials are sometimes sterilized using dry heat. The validation of these dry heat based cycles follows many of the general principles outlined for steam sterilization. Concepts such as equilibration time, temperature mapping, heat penetration, biological lethality and worst-case cycle parameters (shortened times, lower temperatures, faster belt speeds, etc.) are relevant, though in a different context.

It is common practice to determine FH values for dry heat processes in a similar manner to F0 for steam sterilization. However, most applications of dry heat are to depyrogenate and not just to sterilize. The depyrogenation process is not as well understood as the sterilization process. Some evidence indicates that inactivation of endotoxin is a second-order reaction whereas the standard use of FH, D and Z values assumes a single-order reaction. Therefore for depyrogenation cycles there is even more reliance on the biological proof (inactivation of endotoxin) as opposed to only the accumulation of physical data. Even with these limitations it is valuable to collect the FH data to demonstrate reproducibility.

The temperature range allowed in both empty and loaded chamber studies for dry heat cycles is significantly greater than for steam sterilization. In particular, for empty chamber studies, USP 29 states that ± 15ºC is a typical acceptable temperature range when the oven is operating at not less than 250ºC. For loaded chamber studies, a ± 5ºC temperature range is generally achievable.

Depyrogenation processes must be validated to achieve a minimum 3-log reduction of bacterial endotoxin. The endotoxin must be spiked onto the item in a liquid state and allowed to air dry prior to being subject to the depyrogenation cycle. Recovery studies and appropriate controls are a necessary part of these studies.

5.4.1 Dry Heat Ovens

The validation of a dry heat process in an oven, whether it is used for sterilization or depyrogenation, is similar to the validation of a steam sterilization process. Biological indicators, typically Bacillus atrophaeus spores (for sterilization processes) or bacterial endotoxin (for depyrogenation processes) must be used in conjunction with temperature measurements. A successful endotoxin challenge in the validation of a depyrogenation process assures that sterilization has also occurred and there is no need for additional studies with bacterial spores.

The load size and configuration are very important in dry heat processing. These must be carefully designed and duplicated between the validation studies and routine operation.

Blowers/Fans - for proper air balance and the differential pressure between the tunnel and the room environment
Room pressure differentials

5.4.2.1 Empty Tunnel Temperature Distribution

Temperature probes (thermocouples) are distributed across the width of the tunnel and a temperature profile is produced across the conveyor and along the tunnel during operation. A reasonably uniform temperature profile is expected. A probe needs to be located next to the controlling sensor. Note the come-up time to temperature and cool-down times, as these should be consistent in an empty tunnel.

5.4.2.2 Loaded Chamber Heat Penetration

In dry heat tunnel applications heat penetration studies must be performed. Thermocouples must be placed in contact with vials distributed across the tunnel. The temperature of the vial is usually lower than the air temperature.

Test should be performed with all vial sizes. Endotoxin spiked vials should be located adjacent to the penetration thermocouples. Heat penetration studies, conducted as part of the initial validation, must be repeated several times (e.g. three times) to demonstrate consistency. Consider the loading arrangement of the tunnel i.e. at the start, middle and end of a run.

The temperature profile for a specific load should show good reproducibility.

5.4.3 Demonstrating Biological Lethality

The use of BIs to demonstrate biological lethality must be included in the validation approach.

For dry heat sterilization the indicator of choice is Bacillus atrophaeusspores. However, dry heat is also used to depyrogenate. In this case endotoxin (E. coli) is used to validate the depyrogenation activity. If endotoxin is used then Bacillus atrophaeusspore strips are not required as endotoxin is more heat resistant than Bacillus atrophaeus. These biological studies can be performed at the same time as the loaded chamber temperature mapping. If this is done the carriers should be located as close as possible to the thermocouples.

5.4.4 Washing Process for Depyrogenation

In some cases, the depyrogenation of items that cannot be dry heat sterilised (e.g. rubber stoppers) is carried out in a washing/rinsing process. It must be demonstrated that a 3-log reduction in the endotoxin concentration on spiked stoppers can be achieved.

Recovery studies on the spiked stoppers must be performed. The stoppers must be subsequently sterilized using a validated steam sterilization cycle.

5.5 Radiation Sterilization

