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Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container

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This guideline replaces the document *Decision trees for the selection of sterilisation methods* (CPMP/QWP/054/98), which is an annex to the *note for guidance on development pharmaceutics* (CPMP/QWP/155/96); and the document *Decision trees for the selection of sterilisation methods* (EMEA/CVMP/065/99) which is an annex to the *note for guidance: Development pharmaceutics for veterinary medicinal products* (EMEA/CVMP/315/98).

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Guideline on sterilisation of the medicinal product, active substance, excipient and primary container

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Executive summary

Guidance is provided on the selection of appropriate methods of sterilisation for sterile products. Although, terminal sterilisation using a reference condition of the European Pharmacopoeia (Ph. Eur.) is the method of choice whenever possible, this guideline provides information on when other terminal sterilisation processes, sterilising filtration or aseptic processing, (either alone or when combined with an additional post-aseptic processing terminal heat treatment), could be accepted as an alternative.

Guidance is provided on the documentation expected for sterile finished products, sterile active substances, sterile excipients and sterile primary containers (referred to as container in this guideline) in a new marketing authorisation application or a variation application for a medicinal product, (called quality dossier throughout the guideline).

Terminology definitions are included at the end of the document.

1. Introduction (background)

Sterility is a critical quality attribute for all sterile substances, products and containers. Sterility cannot be assured by testing, it needs to be assured by the use of a suitably designed, validated and controlled manufacturing process. Sterility is achieved by controlling several factors such as the bioburden, the sterilisation procedure, the integrity of the container closure system and in the case of aseptic processing, the use of satisfactory aseptic technique.

Terminal sterilisation is preferred to sterilisation by filtration and/or aseptic processing because it is lethal to micro-organisms and a reliable sterility assurance level (SAL) is possible to calculate, validate and control, and thus incorporates a safety margin. For sterile filtration followed by aseptic processing, this is not applicable as accidental contamination caused by inadequate technique cannot be reliably eliminated by monitoring and control. Therefore, terminal sterilisation provides the highest assurance of sterility and should be used whenever possible.

For highly sensitive products, such as most biological products, where terminal sterilisation of the finished product is not possible, sterile filtration and/or aseptic processing under validated and controlled conditions can be accepted.

Sterile filtration and aseptic processing are closely related and difficult to consider separately, since sterile filtration in most cases is followed by at least one aseptic processing step such as filling. In order to focus on the most important aspect of filtration and aseptic processing at each section of this guideline, only one of the two steps may be mentioned, even if both steps are related.

In addition to those finished products where the formulation itself prohibits the possibility of terminal sterilisation, the use of aseptic processing can be accepted in certain situations, even if the formulation itself can be terminally sterilised, if other benefits are gained for patients or users of the product. These situations are specified below in section 4.3.

Container integrity is discussed in ICH Q8, (adopted for human medicinal products only, nevertheless the same principles are also applicable to veterinary medicinal products and containers of sterile substances and containers).

2. Scope

The guideline applies to chemical and biological medicinal products for human and veterinary use but is not applicable to immunological veterinary medicinal products.

It is acknowledged that the recommendations provided for in this guideline may require some adaptation to the specific characteristics of Advanced Therapy Medicinal Products (ATMPs) for human use (e.g. difficulties to differentiate between starting material, active substance and finished product in some cases, scarcity of starting materials/active substance/finished product (autologous products and matched-donor scenario), small volumes of production). The level of documentation that is expected to be included in marketing authorisation applications for ATMPs may be adapted provided that this is justified under a risk-based approach. For veterinary cell based novel therapies, cross reference is made to EMA/CVMP/ADVENT/751229/2016 Questions and Answers on allogenic stem cell-based products for veterinary use: specific questions on sterility.

Guidance is provided on the choice of sterilisation method, the development data and manufacturing data required to demonstrate the suitability of the selected sterilisation process. The same principles (choice of method of sterilisation, development data and manufacturing data) apply to sterile active substances, excipients and primary containers. Only the information expected in the quality dossier, including information related to Good Manufacturing Practice (GMP) certificates, is described. Not all GMP requirements (e.g. environmental monitoring, sterilisation of manufacturing equipment) are referenced in the guideline, only those that are considered specifically relevant for the quality dossier.

The scope of this document includes:

- Terminal sterilisation by steam, dry heat and ionising irradiation using the reference conditions of Ph. Eur. 5.1.1 “Methods of preparation of sterile products” or other conditions stated in that monograph
- Sterilisation by filtration and aseptic processing
- Sterilisation by gas

The concepts in this guideline refer only to absence or removal of bacteria, fungi and bacterial endotoxins. The absence, removal or inactivation of viruses, mycoplasma, prions and other adventitious agents, which could contaminate a product, are not considered. For virus validation reference is made to the Guideline *Virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses, CPMP/BWP/268/95*.

3. Legal basis

This guideline should be read in conjunction with Directive 2001/83/EC on the community code relating to medicinal products for human use, Directive 2001/82/EC on medicinal products for veterinary use as amended and also the current Ph. Eur.

In addition, this guideline should be read in conjunction with all other relevant directives and regulations, and all relevant Commission, (V)ICH and CXMP guidelines, Q&A documents and other documents as linked to or published on the EMA website (www.ema.europa.eu).

4. General requirements

The guideline concerns specific requirements related to sterility, sterilisation processes and aseptic processing of sterile products and product components.

4.1. Requirements for the manufacture of sterile medicinal products and sterile components

The choice of sterilisation method or aseptic processing should be justified, see section 4.3 Selection of sterilisation method.

All sterilisation processes should be carried out according to the instructions of the Ph. Eur. unless justified.

All sterilisation procedures for the finished product, active substance, the excipient(s) or the containers and the name and address of the sterilisation site should be stated. A description of the sterilisation method and/or aseptic processing, including in-process controls and validation data should be provided.

When parametric release of sterility is proposed, the Guideline on real time release testing (formerly Guideline on parametric release), EMA/CHMP/QWP/811210/2009-Rev1 (human products only), the Guideline on Parametric release, EMEA/CVMP/QWP/339588/2005 (veterinary products only) and the text of Ph. Eur. Chapter 5.1.1 should be taken into account.

The bioburden control criteria should be specified prior to all sterilisation processes. High bioburden acceptance criteria should not be justified by the capacity of the sterilisation process or any bioburden reducing step before sterilisation. Acceptance criteria for bioburden are discussed under the relevant sub-sections of 4.1 below.

The levels of bacterial endotoxins in the finished product can be impacted by the bioburden and bacterial endotoxins in the components (i.e. active substance, excipients and containers), and by microbiological contaminants introduced during manufacture. To ensure an acceptable level of bacterial endotoxins in the finished product, the level of microbiological contaminants of the components should be minimal. Acceptance criteria for bioburden and, where relevant, bacterial endotoxins in components and bulk solutions should be specified.

All filters used in the manufacture of the finished product that come in contact with the finished product, or with any component (substance or intermediate product) incorporated in the finished product should be described and the information stated in Table3, section 4.1.5 should be provided in the quality dossier. The information should be in line with the requirements stated in Eudralex GMP Annex 1. For ATMPs, the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products should be followed.

If a secondary container (e.g. secondary pouch for infusion bags or blisters intended to keep the outside of the container sterile) is used to provide a specific protection to the medicinal product, the packaging process should be described, including a risk assessment, since it may affect the sterility of the finished product; for example, trapping moisture between the primary and secondary containers. Information should be provided as to when the packaging step is performed (before or after sterilisation) and any aseptic techniques employed. The proposed processes should be justified from a microbiological perspective. If the use of a secondary container means additional sterilisation of the finished product is performed, this should be justified with regard to sterility assurance and any potential impact on finished product quality.

Documentation regarding sterilisation and aseptic processing to be included in the quality dossier is presented below. The documentation could, for practical reasons, be presented in connection with the item which is to be sterilised if a reference to the location of the documents is provided in section 3.2.P.3.3 or in Part 2 B. The documents may be provided for human products in sections 3.2.S.2 Manufacture, 3.2.P.2 Pharmaceutical development, 3.2.P.3 Manufacture, 3.2.P.4 Control of excipients,

or 3.2.P.7 Container closure system, or for veterinary products in Part 2 A.4 Development pharmaceutics, Part 2 B.1 Manufacturing method, Part 2 C.1 Active substance, Part 2 C.2 Excipients or Part 2 C.3 Container closure systems. The documentation should be provided for all sites performing sterilisation or aseptic processing, regardless of whether the processes are performed in-house or outsourced.

Process parameters such as processing and holding times are assessed and agreed during the evaluation of the quality dossier. These may be further reviewed during GMP inspections, which may result in changes to the registered dossier being required.

4.1.1. Steam sterilisation

All steam sterilisation processes require a minimum lethality of $F_0 \geq 8$ minutes and a minimum process hold temperature of 110 °C.

Sterilisation processes of different levels of lethality are presented in Table 1, along with the documentation to be included in the quality dossier. The processes in the table are presented with decreasing lethality when read from top to bottom, thus the first feasible process should be selected.

For sterilisation using a reference condition of the Ph. Eur. 5.1.1 (≥121 °C, ≥15 min in all units) validation data for the sterilisation cycle is not required to be submitted in the quality dossier.

If used as an additional control to measure the process lethality, F_0, should be stated, together with the lowest temperature measured by the temperature sensors to determine F_0.

Steam sterilisation performed with finished product temperature below 115 °C during the holding phase is an exceptional case and should be scientifically justified and supported by additional data as described in Table 1. If temperatures below 110 °C are included (during heat-up and cool-down) in the determination of F_0, this should be justified.

Information regarding the F_0 concept and microbial reduction is provided in Ph. Eur. 5.1.5 *Application of the F_0 concept to steam sterilisation of aqueous preparations*.

The bioburden limit should be in line with any pre-sterilisation bioburden reduction process capability (e.g. filtration). For aqueous solutions, the limits stated in Table 1 are acceptable for active substances and drug product formulations without further justification. Other testing regimes and limits to control bioburden at the defined level should be justified.

Moist heat processes with an $F_0 < 8$ min may be suitable as a post-aseptic processing terminal heat treatment for formulations that cannot withstand a complete terminal sterilisation cycle. Such processes may further ensure a SAL of sterile filtered (or otherwise sterilised) bulk components, which have been aseptically filled. Post-aseptic processing terminal heat treatments are also presented in Table 1.

It is emphasised that this additional post-aseptic processing terminal heat treatment should not compensate for poor aseptic manufacturing practice. The same requirements for the aseptic part of the process apply as for finished products manufactured without such an additional post-aseptic processing terminal heat treatment.

Table 1 Cycles for steam sterilisation and post-aseptic processing terminal heat treatment and corresponding data required in the quality dossier

Cycle	Type of process	Information in dossier*	Bioburden level before steam sterilisation or terminal heat treatment	Bioburden Characterised	Process hold temperature
Ph. Eur. 5.1.1 Reference Cycle	Sterilisation	1, 6	100 CFU/100ml (non-routine)	No	≥ 121 °C for ≥15 minutes
Overkill cycle F_o >12 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (non-routine)	No	≥ 121 °C
F_o > 8 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (routine)	No	> 115 °C
F_o > 8 min	Sterilisation	1, 2, 3, 5, 7, 8	100 CFU/100ml (routine)	Yes**	> 115 °C
F_o > 8 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (routine)	Yes	> 110 °C
F_o > 8 min	Sterilisation	1, 2, 3, 5, 7, 8	100 CFU/100ml (routine)	Yes**	> 110 °C
F_o <8 min	Post-aseptic processing terminal heat treatment	1, 2, 3, 4, 7, 8	0 CFU/100ml, aseptic filtration and processing prior to terminal heat treatment (routine)	Yes***	> 110 °C****
F_o <8 min	Post-aseptic processing terminal heat treatment	1 2, 3, 5, 7, 8	0 CFU/100ml, aseptic filtration and processing prior to terminal heat treatment (routine)	Yes***	> 110 °C****

* For clarification of the code numbers, see below

** In-process control demonstrating acceptable heat resistance of bioburden

*** The bioburden prior to the sterilisation step (i.e. filtration) should be characterised for heat resistance

**** Temperatures below 110 °C may be used if justified. The requirement for additional documentation for such cycles is evaluated on a case by case basis

Clarification of the information to be presented in the quality dossier

1: Sterilisation time, temperature profile

2: Sterilisation method (for instance saturated steam cycle, air/steam-overpressure cycle, vacuum phase) description including SAL

3: Validation of F_{0Phys} and F_{0Bio}

4: Biological indicator with a $D_{121} \geq 1.5$ minutes used in the validation

5: Biological indicator with a $D_{121} < 1.5$ minutes used in the validation

6: No validation data requested in the dossier, only a confirmation that validation has been performed.

7: Validation data to be provided in the dossier is presented below

8: Additional validation data to be provided in the dossier is presented below

Validation data to be provided in the quality dossier for all steam sterilisation processes that do not fulfil the requirements of Ph. Eur. 5.1.1 standard process (required information 7 in Table 1):

- Load mapping of the chamber and load mapping distribution of the items in the chamber (including the slowest to heat locations); summary or confirmation of performance.
- Physical and biological cycle effect confirmation summary of at least three sterilisation runs demonstrating an SAL $\leq 10^{-6}$, as described in Ph. Eur. 5.1.1 ensuring:
  - Demonstration that the sterilisation load in the steriliser chamber achieves the specified cycle parameters, including time, temperature, pressure and F_0, if applicable;
  - Acceptable temperature differences between temperature sensors in the load;
  - Acceptable F_0 variability within the load;
  - Relationship between physical and biological validation.

  For the biological validation, a biological indicator as described in Ph. Eur. chapter 5.1.2 *Biological indicators and related microbial preparations used in the manufacture of sterile products* with a D_{121}-value of ≥1.5 minutes should be used.

  The SAL should be determined, its microbiological basis should be justified and details of calculations provided in the quality dossier. Preferably it should be calculated from the maximum bioburden per container and the D-value of the biological indicator used in the validation.

Additional validation data to be provided in the quality dossier for low energy steam processes or where a bio-indicator with a D_{121}-value of <1.5 minutes is used in the validation of the sterilisation process (required information 8 in Table 1):

The following additional data should be provided:

- A justification for the start point of the sterilisation phase, that is the temperature when the temperature sensors record the F_0 from the start to end of the process;
- Biological indicators with suitable resistance at the actual temperature range as described in Ph. Eur. 5.1.2 should be included in the validation to demonstrate sensitivity to the process.

More detailed validation data is requested to ensure that the proposed sterilisation process is suitable for low temperature processes and for processes using biological indicators of low heat resistance because:

- The change in lethal effect in relation to the process temperature may not be log linear at lower sterilisation temperatures.
- The SAL demonstrated in the validation of a sterilisation process is dependent on the heat resistance of the biological indicator used in the validation of the process. When a biological indicator of low D-value is used in the validation of the sterilisation process, the SAL demonstrated becomes numerically higher, but does not provide as high a safety margin as where a more resistant biological indicator is used. The SAL should always be established in relation to a D-value that is higher than that of the normal bioburden at routine production.

4.1.2. Dry heat sterilisation

Time and temperature of the sterilisation cycle and a bioburden limit should always be stated.

For sterilisation using a reference condition of the Ph. Eur. 5.1.1 (a minimum of 160 °C for at least 2 h), the validation data for the sterilisation cycle is not required to be submitted in the quality dossier. For sterilisation cycles with time and/or temperature lower than the reference conditions of the Ph. Eur., physical and biological validation of the sterilisation cycle should be provided to demonstrate an SAL of $\leq 10^{-6}$, as described in Ph. Eur. 5.1.1. The SAL of such a sterilisation process should be calculated from the maximum bioburden per container.

Where required, sufficient validation data should be submitted to demonstrate that an SAL of $\leq 10^{-6}$ is obtained for all containers. The data submitted should include at least, but is not limited to:

- Load mapping of the chamber and load mapping distribution of the items in the chamber (including the slowest to heat locations) – summary or confirmation of performance;
- Physical and biological cycle effect confirmation summary of at least three sterilisation runs ensuring:
  - Demonstration that the sterilisation load in the steriliser chamber achieves the specified cycle parameters, including time, temperature, and, lethality;
  - Acceptable temperature differences between temperature sensors in the load;
  - Acceptable lethality variability within the load;
  - Relationship between physical and biological validation.

For the biological validation, a biological indicator as described in Ph. Eur. chapter 5.1.2 should be used.

A maximum bioburden limit of 100 CFU/100 g or 100 CFU/100 ml would be acceptable for parenteral finished product formulations without further justification. For active substances and finished products that are not used for parenteral administration, a maximum total bioburden limit of 10 CFU/g or 10 CFU/ml is acceptable without further risk based justification. Other testing regimes and limits to control bioburden at the defined level should be justified. A justified bioburden limit should also be established for empty containers.

Dry heat at temperatures of greater than 220 °C for a validated time is frequently used for both sterilisation and depyrogenation of glassware and other heat-resistant container materials e.g. aluminium crimps. In this case, demonstration of a 3 log reduction in heat-resistant endotoxins can be used as validation criteria.

4.1.3. Ionization radiation sterilisation

For this method of sterilisation, the reference absorbed dose is ≥25 kGy. Other doses may be used to achieve an SAL $\leq 10^{-6}$, if justified and validated.

Data as requested in Note for Guidance “The use of Ionization Radiation in the Manufacture for Medicinal Products” and in compliance with Ph. Eur. chapter 5.1.1 should be provided. Relevant guidance in establishing the radiation dose other than 25 kGy is available in ISO standard 11137.

Where any requirements in ISO 11137 are in contradiction to requirements stated in any Note for Guidance issued by the EMA or Ph. Eur. monograph, the requirements of the Ph. Eur. and the Note for guidance apply.

4.1.4. Gas sterilisation

4.1.4.1 General considerations

Generally, gas sterilisation is only acceptable if no other method of sterilisation is possible. Gas sterilisation provides sterilisation of the surface of materials. It is mainly employed for sterilising packaging materials and equipment, and has therefore only been included in the decision tree for containers. To ensure adequate sterility, sufficient penetration by gas and moisture is essential. This should be followed by a purging process to ensure that any residues of gas or related transformation by-products are below concentrations that could give rise to toxic effects during use of the finished product. The effectiveness of the purging process should be demonstrated.

Gas sterilisation of porous compounds, such as dry powders, is not acceptable unless other methods of sterilisation are not feasible and its use is scientifically justified. Prior to the gas sterilisation, the active substance or excipient should be sterile filtered and crystallised under aseptic conditions to minimise bioburden and entrapment of micro-organisms within the crystals. Convincing evidence should be provided demonstrating that the material to be sterilised is not susceptible to compression preventing gas and moisture penetration during sterilisation.

A description of the apparatus, quantitative data on gas(es) to be used, the bioburden prior to sterilisation, the time of exposure to the gas, the temperature and humidity prior to and during each step of the sterilisation cycle, and, if applicable, the conditions for the removal of any toxic gas residues should be provided. Humidity used for the preconditioning and/or conditioning of the material to be sterilised shall be generated by clean steam. These conditions should be monitored by appropriate in-process controls with justified acceptance criteria. The process should be developed and validated in compliance with Ph. Eur. 5.1.1 and 5.1.2. A risk assessment with regards to residual toxic impurities should be conducted and a control strategy should be provided where applicable. The requirements should be in accordance with the requirements of ICH M7 “Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk”. Even if the relevant product is outside the scope of that guideline, its limits for highly toxic impurities could be applied.

Results of the process validation should demonstrate an SAL of $\leq 10^{-6}$.

The effectiveness of the process should be routinely checked for every batch confirming that the process parameters and biological indicators are all within their acceptance criteria and by sterility testing. Parametric release is not acceptable for gas sterilisation (according to Ph. Eur. chapter 5.1.1).

4.1.4.2 Ethylene oxide sterilisation

Ethylene oxide (ETO) sterilisation processes should be developed and validated in compliance with Ph. Eur. 5.1.1 and 5.1.2. Relevant guidance in establishing the sterilisation process cycle parameters and validation is available in ISO standard 11135.

ETO is a gas which is highly toxic. ETO sterilisation is generally only acceptable if no other method of sterilisation is possible. The risk assessment should consider the residual known genotoxic impurities (such as ETO and halogenated ethylenehydrines). This should be evaluated in accordance with the requirements of ICH M7 “Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk”, unless the relevant product is outside the scope of that guideline. For products outside the scope of ICH M7, the applicant should apply limits for highly toxic impurities in accordance with ICH M7, or the acceptance criteria stated in Table 2, whichever is most appropriate.

For empty containers intended to be filled with aqueous products, (e.g. prefilled syringes), the need to justify the use of ETO in the sterilisation of the container prior to filling can be waived, as the degradation kinetics of ETO in an aqueous medium have been sufficiently demonstrated. However, the levels of toxic residues (ETO and halogenated ethylenehydrines) in the finished product need to fulfil the requirements of ICH M7, or the limits stated in Table 2 below, as applicable.

Table 2 Limits for toxic gas residues from ethylene sterilisation where the ICH M7 limits do not apply

Material	Ethylene oxide	Ethylene chlorhydrin (or any other halogenated ethylenehydrine)
Raw materials	1 µg/g	50 µg/g
Finished product (when used on the finished product)	1 µg/g	50 µg/g
Container (based on simulated use)	1 µg/ml	50 µg/ml

4.1.5. Sterile filtration

The filter data to be provided in the quality dossier is summarised in Table 3.

Table 3 Filter data to be provided in the quality dossier for filters in contact with the drug product or components of the drug product

Parameter	Filter		Comment	
	Non-sterilising[1]	Sterilising[1]		
General information on filter				
Type of material, nominal pore size	X	X		
Number of filters	X	X		
Filter area	-	X		
Filter integrity test	-	X	Principle of the test, details on when the tests are performed, solution(s) used in the test and acceptance criteria before and after filtration should be described.	
Filter validation			Solution used	Comment
Potential sorption of solution components to filter	X	X	Product	
Solution Compatibility	X	X	Product	Worst case conditions with regards to for instance sterilisation process, contact time, filtration time, pressure, filtered volume.
Filter retention capacity	-	X	Product [2]	Minimum 10^7 CFU/cm^2 using a justified indicator organism and the actual solution.
Filter integrity test limits	-	X	Product [3]	
Extractable and leachable substances from the filter	X	X	Product [4]	Justified surrogate solution may be used.

[1] As defined in GMP, Annex 1
[2] Validation of filter retention capacity may be combined with solution compatibility. If the product solution affects the indicator organisms negatively, it should be neutralised before adding the organisms. For validation, a suitable challenge microorganism representing the worst-case challenge to the filter should be used.
[3] If the test is performed using a different solution in routine manufacture (for instance water for injections), the limits should be established in this solution.
[4] Data on leachables is relevant only if the extractables data indicate that toxic components may leach into the solution to be filtered.

The integrity of the sterilised filter should be verified by testing before use unless specifically justified and validated, and should be verified by on line testing immediately after use. Nominal pore sizes of 0.22 µm or less are acceptable without further justification, in accordance with Ph. Eur.

For routine commercial manufacturing, bioburden testing should be performed on the bulk solution immediately before sterile filtration.

In most situations, a limit of NMT 10 CFU/100 ml (TAMC) would be acceptable for bioburden testing. If a pre-filter is added as a precaution only and not because the unfiltered bulk solution has a higher bioburden, this limit is applicable also before the pre-filter and is strongly recommended from a GMP point of view. A bioburden limit of higher than 10 CFU/100 ml before pre-filtration may be acceptable if this is due to starting material known to have inherent microbial contamination. In such cases, it should be demonstrated that the first filter is capable of achieving a bioburden of NMT 10 CFU/100 ml prior to the last filtration. Bioburden should be tested in a bulk sample of 100 ml in order to ensure the

sensitivity of the method. Other testing regimes to control bioburden at the defined level should be justified.

The maximum time between the start of bulk solution preparation and sterile filtration should be stated, minimised and appropriately supported by data. Filtration times longer than 24 hours should be justified.

If a sterile filtered bulk solution is not filled into the final product containers within 24 hours, the sterile filtration should, unless justified, be repeated immediately before filling. An additional bioburden test should be performed before any further bioburden reduction step after the holding time. The holding time should be adequately justified.

4.1.6. Aseptic processing

Aseptic processing is not considered to be a sterilisation process but concerns the usage of technologies to process sterile components avoiding addition of microbiological contaminants, e.g. use of an isolator or Restricted Access Barrier System (RABS).

For aseptic processing, information on the bulk holding time before filling and on the filling time should be stated and appropriately supported by data. The times should be minimised. The grounds for holding and filling times longer than 24 hours should be justified and supported by a risk assessment. It should be verified that the results of the media simulations support the proposed holding and processing times. The actual results of media simulations fall within the field of GMP and need not be presented routinely, but may be requested by the competent authorities in certain circumstances since such data are important to justify proposed holding and filling times.

Sterile containers should be used for aseptically treated active substances, excipients and finished products.

Where blow-fill-seal technology is used for aseptically treated products, a summary of the validation data should be provided to confirm that the container produced is sterile. The validation should, using a biological indicator with a suitable resistance, demonstrate a SAL of $\leq 10^{-6}$ for the surface of the container. The bioburden of the material(s) used for the manufacture of the blow-fill-seal container should be controlled. The limit should be justified in relation to the lethality of the validated blow-fill-seal process. The bioburden limit should also include a safety margin as a precaution for any possible bioburden enclosed within the material.

The majority of ATMPs cannot be terminally sterilised. In such cases, the manufacturing process should be conducted aseptically. Further details on aseptic manufacturing for ATMPs can be found in the Guidelines on Good Manufacturing Practice for Advanced Therapy Medicinal Products.

4.2. Good manufacturing practice for sterile active substances, sterile excipients and sterile containers

Volume 4 of "The rules governing medicinal products in the European Union" contains guidance for the interpretation of the principles and guidelines of good manufacturing practices for medicinal products for human and veterinary use laid down in Commission Directives 91/356/EEC, as amended by Directive 2003/94/EC, and 91/412/EEC respectively. For Advanced Therapy Medicinal Products, the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products should be followed.

4.2.1. Active substances

The basic GMP requirements for active substances used as starting materials (European Union (EU) GMP guide part II) apply to the manufacture of sterile active substances up to the point immediately prior to the active substance being rendered sterile. The sterilisation and aseptic processing of sterile active substances are not covered by EU GMP Part II but should be performed in accordance with the principles and guidelines of GMP as laid out in the relevant EU Directive and interpreted in the GMP Guide including its Annex 1.

The sterilisation and aseptic processing of active substances is considered to be a step in the manufacture of the medicinal product. This implies that for any active substance manufacturer who performs sterilisation and subsequent aseptic handling of the active substance, a valid manufacturing authorisation or GMP certificate from an EEA authority or from an authority of countries where mutual recognition or other Community arrangements apply has to be submitted.

The same GMP and data requirements also apply to sterile active substances supported by a Certificate of Suitability issued by the European Directorate for the Quality of Medicines & HealthCare (EDQM) or described in an Active Substance Master File (ASMF).

4.2.2. Excipients

All the excipient sterilisation sites should be stated by name and address in the dossier.

For excipients required to be sterile (i.e. those subsequently used in an aseptic manufacturing process), the site where sterilisation of the excipients takes place may not have undergone inspection by an EU authority and consequently may not hold an EU GMP certificate in relation to this activity. Nevertheless the sterilisation of an excipient is a critical process and the sterility of the excipient is a critical quality attribute to ensure the sterility of the finished product. When a GMP certificate is not available, a statement should be provided confirming that the finished product manufacturer has evaluated all the manufacturers of sterile excipients with regards to their quality system related to the sterilisation of the excipient. For products for human use this evaluation should be conducted in line with the (GMP) Guidelines of 19 March 2015 on the formalised risk assessment for ascertaining the appropriate good manufacturing practice for excipients of medicinal products for human use by taking into account the specific requirements of Annex 1 of EU GMP-Guidelines.

4.2.3. Containers

For containers required to be sterile (i.e. those subsequently used in an aseptic manufacturing process), the site where sterilisation of the containers takes place may not have undergone inspection by an EU authority and consequently may not hold an EU GMP certificate in relation to this activity[1]. When a GMP certificate is not available, certification that the sterilisation has been conducted and validated in accordance with the following ISO standards would be considered sufficient to provide an acceptable level of sterility assurance for the empty container:

1. I.S. EN ISO 20857 *Sterilization of Health Care Products - dry Heat - Requirements for the Development, Validation and Routine Control of a Sterilization Process for Medical Devices;*
2. I.S. EN ISO 11135 *Sterilization of Health-care Products - Ethylene Oxide - Requirements for the Development, Validation and Routine Control of a Sterilization Process for Medical Devices;*

[1] Sites located in the EU which perform sterilisation of primary containers only are not required to hold a Manufacturer's/Importer's Authorisation (MIA). Sites located in the EU, which carry out sterilisation of medicinal products, are required to hold a MIA in relation to these activities.

3. I.S. EN ISO 17665-1 *Sterilization of Health Care Products - Moist Heat - Part 1: Requirements for the Development, Validation and Routine Control of a Sterilization Process for Medical Devices, and, ISO/TS 17665-2 Sterilization of health care products -- Moist heat -- Part 2: Guidance on the application of ISO 17665-1*;
4. I.S. EN ISO 11137-1 *Sterilization of Health Care Products - Radiation - Part 1: Requirements for Development, Validation and Routine Control of a Sterilization Process for Medical Devices*;
5. I.S. EN ISO 11137-2 *Sterilization of Health Care Products - Radiation - Part 2: Establishing the Sterilization Dose*;
6. I.S. EN ISO 11137-3 *Sterilization of Health Care Products - Radiation - Part 3: Guidance on Dosimetric Aspects*.

It is the responsibility of the manufacturer of the medicinal product, to ensure the quality, including sterility assurance, of containers. The site where QP certification of the finished product takes place, and other manufacturing sites which are responsible for outsourcing this sterilisation activity, should have access to the necessary information to demonstrate the ongoing qualification status of suppliers of this sterilisation service. This may be checked during inspections of the manufacturer of the finished product. The Competent Authorities may also decide, based on risk, to carry out their own inspections at the sites where such sterilisation activities take place.

Quality Dossier requirements

The following details regarding the sterilisation of the container components should be included in the quality dossier:

1. The sterilisation method and sterilisation cycle;
2. Validation of the sterilisation cycle if the sterilisation cycle does not use the reference conditions stated in the Ph. Eur.;
3. The name and address of the site of sterilisation and, where available*, details of GMP certification of the site.

*Where the container component is a CE-marked Class Is sterile device (e.g. sterile syringe), a declaration from the device manufacturer that the component is a Class Is sterile device, together with a copy of the certificate of conformity from the Notified Body will suffice. In the absence of a GMP certificate or declaration that the component is a CE-marked Class Is medical device, confirmation by finished product manufacturer that the sterilisation process has been conducted and validated in accordance with the relevant ISO standards should be provided.

4.3. Selection of sterilisation method

Finished products intended to be sterile should be terminally sterilised in their final container whenever possible, as clearly stated in the Ph. Eur., general chapter 5.1.1. Similarly, active substances, excipients and containers when required to be sterile should be packed before they are sterilised whenever possible. When terminal sterilisation by heat is not possible, the application of an alternative method of terminal sterilisation, sterilising filtration and/or aseptic processing may be considered. It is recognised that terminal sterilisation processes utilising conditions other than the Ph. Eur. reference conditions may be developed to provide satisfactory SALs and such alternative processes may be acceptable when properly designed, validated and controlled.

If a sterilisation process using principles other than those described in the Ph. Eur. (steam, dry heat, ionising radiation, gas sterilisation and sterilising filtration) is intended to be used for the sterilisation of an active substance, excipient, container or finished product, the applicant may consider seeking scientific advice regarding the acceptability of the method and the documentation required.

During the manufacturer's evaluation of whether a terminal sterilisation cycle is possible, substantial efforts should be made to enable terminal sterilisation. If the active substance or another component of the finished product is shown to degrade significantly or an impurity limit is exceeded during shelf-life under even the least stressful terminal sterilisation conditions, the efforts made to develop a formulation and container capable of undergoing terminal sterilisation should be presented in the development section. Such efforts could be selection of optimal pH, choice of excipients (qualitative and quantitative), container, optimisation of sterilisation method and manufacturing conditions.

In case of medicinal products containing highly sensitive active substances, (e.g. proteins or other heat labile biological substance), where it is well known that terminal sterilisation is not possible, a justification based on a scientific rationale is generally acceptable and further justification of the choice of aseptic processing discussed later in section 4.3 may not be needed.

The principles for the choice of sterilisation process for finished products and containers are presented in the form of decision trees in section 5 of this guideline. The principles of the decision trees may also be applied for the sterilisation of active substances and excipients.

For finished products where terminal sterilisation is not possible and aseptic processing is proposed, the decision trees should be applied to individual components or mixtures of components in the formulation. An impact on the shelf-life or storage conditions caused by a terminal sterilisation process is not in itself a reason to exclude terminal sterilisation, unless the new storage condition or shelf-life would cause significant problems for the user.

Terminal sterilisation should not be ruled out purely on the basis of an increase in degradation products above the qualification thresholds in ICH Q3A/VICHGL10 (active substances), ICH Q3B/ VICH GL11 (finished products) or the impurity limits in ICH M7 for products in the scope of that guideline without additional justification. If impurities are either metabolites or are generated at levels already qualified, then terminal sterilisation is still considered feasible. However, if the degradation products are not qualified at the level at which they occur, then sterile filtration and aseptic processing may be selected. For medicinal products for human use impurities which occur above the identification threshold should be specified in the finished product specification.

The risk induced by the degradation should be balanced by the risk induced with an aseptic manufacturing method, also taking in account the posology of the finished product and the nature of the degradation products. Attempts to find terminal sterilisation conditions adjusted to give acceptable impurity levels based on degradation mechanisms of the active substance and the actual bioburden should be described in the quality dossier.

In certain cases, as described in the bullet points below, the use of aseptic processing may be accepted, even if the formulation itself can be terminally sterilised. The approach should be clearly documented, explained and scientifically justified. Such cases could be justified by:

- User benefit provided by a container that cannot be terminally sterilised such as:
  - Eye drop containers enabling administration of single drops to the eye;
  - Containers enabling non parenteral multi-dose preservative free medicinal products for human use;

- Enhanced ease of administration;
- Safer handling of toxic products, for instance plastic vials instead of glass vials for cytotoxic medicinal products.

The choice to use a heat-labile container cannot in itself be the sole reason for not applying a terminal sterilisation process and alternative materials should be investigated. Thus, a discussion regarding the efforts made to develop a container that may be terminally sterilised should be included.

- Enabling as long a shelf-life as possible for radiopharmaceutical medicinal products with a shelf-life of less than one week.

The acceptability of aseptic processing should be based on the application of the decision tree and a risk assessment. The bullet points below are not intended to be used to justify aseptic processing as such, but are only intended to provide guidance on issues that are considered when evaluating the acceptability of a sterilisation or aseptic processing. Considerations include (but are not limited to):

- Evidence that the proposed container with enhanced user benefits is fit for purpose;
- Stability of the active substance, the degradation mechanism(s) and the toxicity of impurities formed during the sterilisation process;
- The volume to be administered per dose.

In conclusion, the justification for the chosen sterilisation or aseptic processing should include a thorough benefit risk evaluation and it should be demonstrated that suitable development efforts have been made.

For advanced therapy medicinal products, the microbiological quality of all components, process equipment and the aseptic techniques of the manufacturing processes are of utmost importance when the finished product cannot be sterilised. For those medicinal products that cannot be sterilised, such as cell based medicinal products, a detailed risk assessment with regards to microbial contamination should be provided. A risk based approach is already foreseen for these ATMP (see Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products, EMA/CAT/CPWP/686637/2011).

5. Decision trees

The decision trees in Figures 1 and 2 are intended to assist in the selection of the optimal sterilisation method taking into account the various issues to be considered. When moving down the decision trees, the methods generally show a decreasing assurance of sterility and therefore, the first feasible option should normally be chosen.* The decision trees have been elaborated primarily for finished products containing chemical active substances, but may be applicable also to other types of products (including active substance and excipients). Figure 3 provides the corresponding information for empty containers. The decision tree is not applicable to sterile empty containers that are CE marked medical devices. In the case of biological products, an alternative approach may be appropriate.

*While sterilisation by heat and sterilisation by ionising irradiation provide the same assurance of sterility, sterilisation by heat has lower risk (e.g. radiolysis impurities) and is more easily controlled than sterilisation by ionising irradiation. For these reasons, heat is given priority over ionising irradiation in the decision trees.

Figure 1 Decision tree for sterilisation choices for aqueous products

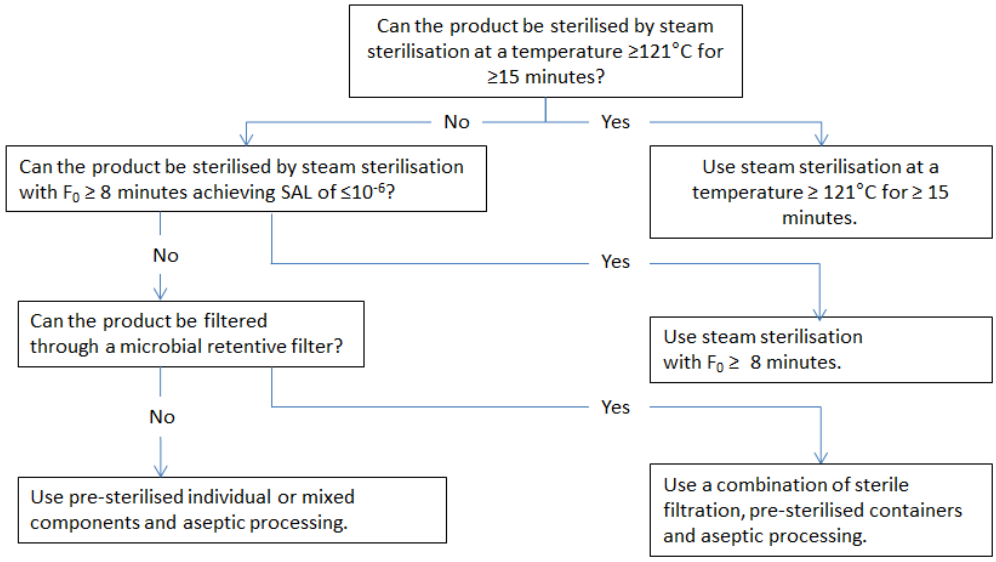


Figure 2 Decision tree for sterilisation choices for dry powder products, non-aqueous liquid or semi-solid products

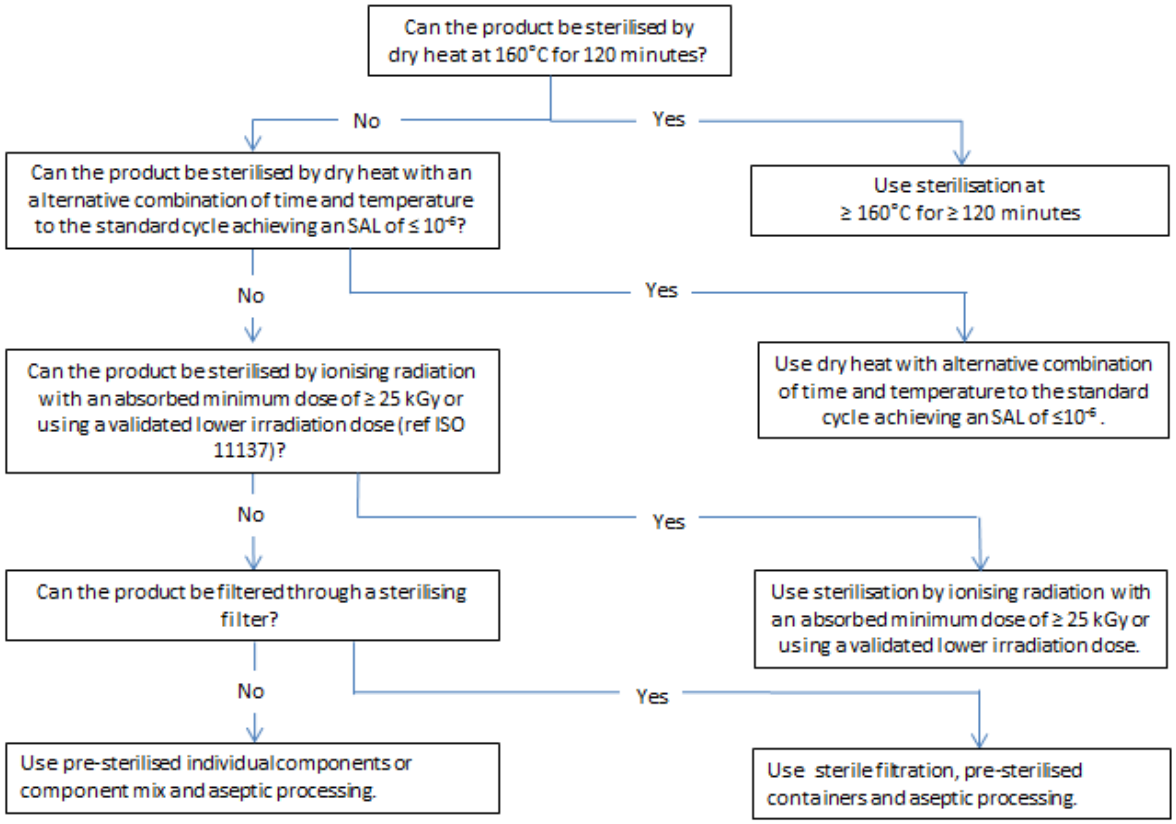
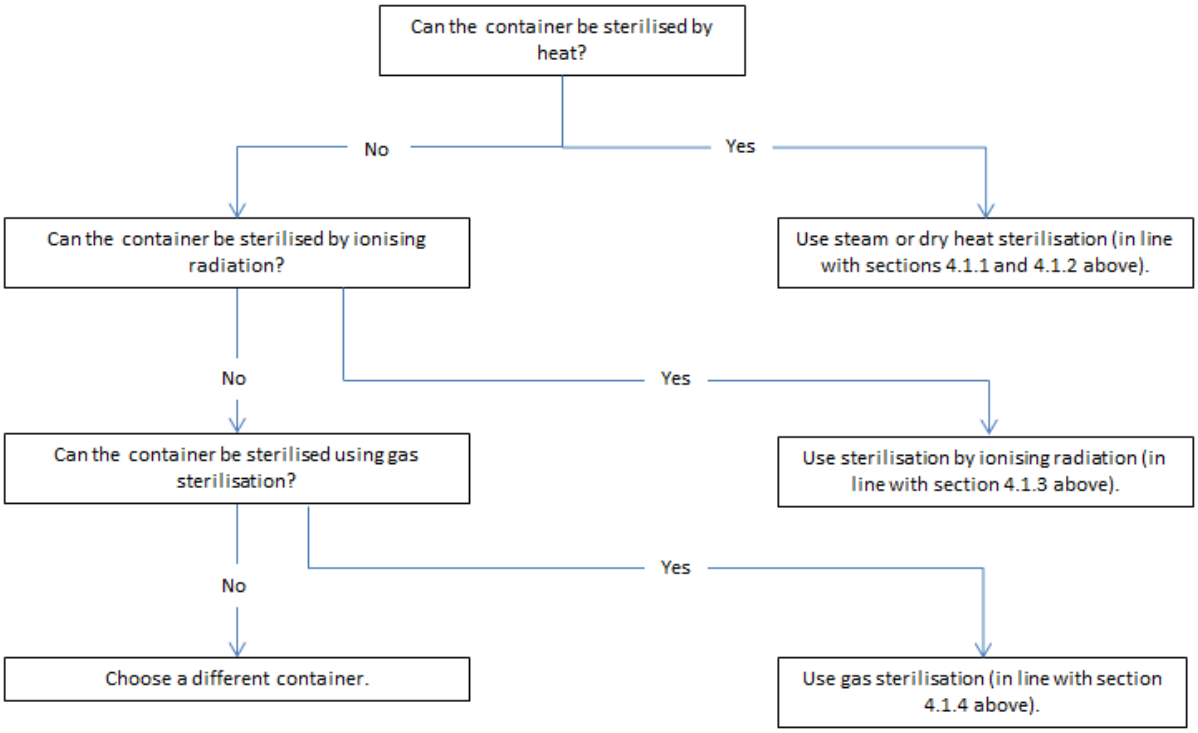


Figure 3 Decision tree for sterilisation choices for containers



6. Definitions

Aseptic processing	A process performed maintaining the sterility of a product that is assembled from components, each of which has been sterilised by steam, dry heat, ionizing radiation, gas or sterile filtration. This is achieved by using conditions and facilities designed to prevent microbiological contaminants.
Bioburden	The total number of micro-organisms associated with a specific item prior to any sterilisation or bioburden reduction step.
Biological indicator	Biological indicators are test systems containing viable microorganisms (usually spores of bacteria) that provide a defined challenge to verify the required effectiveness of a specified sterilisation process.
Colony Forming Unit (CFU)	A microbiological term that describes the formation of a single macroscopic colony after the introduction of one or more micro-organisms to microbiological growth media. One colony forming unit is expressed as 1 CFU.
Critical Quality Attribute	A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate acceptance criteria, range, or distribution to ensure the desired product quality
Depyrogenation	A process used to destroy or remove pyrogens (e.g. endotoxins).
D-value (decimal reduction value)	The value of a parameter of sterilisation (duration or absorbed dose) required to reduce the number of viable organisms to 10 per cent of the original number. It is only of significance under precisely defined experimental conditions. D_{121} is the D-value of the relevant spores at 121 °C.
F_0 value	The F_0 value of a saturated steam sterilisation process is the lethality expressed in terms of the equivalent time in minutes at a temperature of 121 °C delivered by the process to the load in its container with reference to micro-organisms possessing a theoretical Z-value of 10.
Filling time	The time used to fill a bulk product into containers until the container is closed or, in the case of a product which is lyophilized after the

	filling, until the lyophilisation chamber is closed.
Holding time	The time between two process steps.
Immunological veterinary medicinal product	A veterinary medicinal product administered to animals in order to produce active or passive immunity or to diagnose the state of immunity.
Lethal (process)	A process that kills the microorganisms exponentially.
Overkill sterilisation	A process with a lethality of F_{0BIO} > 12 minutes. For example a process that provides at least a 12 log reduction of biological indicator micro-organisms having a minimum D value of 1 minute.
Ph. Eur. sterilisation reference conditions	The reference conditions for sterilisation specified in Ph. Eur. 5.1.1, i.e. terminal steam sterilisation at ≥121 °C for 15 min, terminal dry heat sterilisation at ≥160 °C for ≥2 h or terminal ionising radiation of 25 kGy.
Post-aseptic processing terminal heat treatment	A terminal moist heat process employed after aseptic processing which has been demonstrated to provide a SAL ≤10^{-6}, but where the requirements of steam sterilisation (for example, F_0≥8 min) are not fulfilled.
SAL	Sterility Assurance Level. The SAL for a given sterilisation process is expressed as the probability of micro-organisms surviving in a product item after exposure to the process. An SAL of 10^{-6}, for example, denotes a probability of not more than 1 non-sterile item in 1 × 10^6 sterilised items of the final product.
Slowest to heat locations	Location in the load that remains coldest or where the temperature is raising slowest during the sterilisation process. It could, in a figurative sense, also be used for other sterilisation methods for the location in the load achieving the lowest level of sterilising energy.
Steam sterilisation	Reference is made to the description in Ph. Eur. 5.1.1.
Sterilisation	A suitably designed, validated and controlled process that inactivates or removes viable micro-organisms in a product until sterility is obtained.

Sterility	Sterility is the absence of viable microorganisms, as defined by a sterility assurance level equal to or less than 10^{-6}. The inactivation of microorganisms by physical or chemical means follows an exponential law; thus there is always a finite statistical probability that a micro-organism may survive the sterilising process. For a given process, the probability of survival is determined by the number, types and resistance of the microorganisms present and by the environment in which the organisms exist during treatment.
TAMC	*Total aerobic microbial count:* The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using casein soya bean digest agar.
Terminal process	A process where a finished product is processed in its primary container, for example terminal sterilisation or post-aseptic processing terminal heat treatment.
Validation	Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.
Worst case	A set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, that pose the greatest chance of process or product failure (when compared to ideal conditions). Such conditions do not necessarily induce product or process failure.
z-value	The z-value is the change in temperature required to alter the D-value by a factor of 10.

7. References

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Note for Guidance: Development Pharmaceutics for veterinary medicinal products: Decision tree for the selection of sterilisation methods, EMEA/CVMP/065/99;

Note for guidance on manufacture of the finished dosage form, CPMP/QWP/486/95;

Note for Guidance: Manufacture of the finished dosage form, EMEA/CVMP/126/95;

ICH guideline Q8 (R2) on pharmaceutical development, EMA/CHMP/ICH/167058/2004;

European Pharmacopoeia general chapter 5.1.1 'Methods of preparation of sterile products';

Note for Guidance: Virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses, EMA/CPMP/BWP/268/95;

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, as amended;

Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products, as amended;

Guideline on real time release testing (formerly Guideline on parametric release), EMA/CHMP/QWP/811210/2009-Rev1;

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European Pharmacopoeia general chapter 5.1.5 'Application of the F_0 concept to steam sterilisation of aqueous preparations';

European Pharmacopoeia general chapter 5.1.2 'Biological indicators and related microbial preparations used in the manufacture of sterile products';

NfG on The use of Ionisation Radiation in the Manufacture of Medicinal products 3AQ4A;

EN/ISO 11137, Sterilisation of health care products – Radiation;

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I.S. EN ISO 11135 Sterilization of health-care products - Ethylene Oxide - Requirements for the development, validation and routine control of a sterilization process for medical devices I.S. EN ISO

17665-1 Sterilization of health care products - Moist heat - Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices

ISO/TS 17665-2 Sterilization of health care products -- Moist heat -- Part 2: Guidance on the application of ISO 17665-1

I.S. EN ISO 11137-1 Sterilization of health care products - Radiation - Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices

I.S. EN ISO 11137-2 Sterilization of health care products - Radiation - Part 2: Establishing the sterilization dose

I.S. EN ISO 11137-3 Sterilization of health care products - Radiation - Part 3: Guidance on dosimetric aspects of development, validation and routine control

ICH Q3A (R2) Impurities in new drug substances, CPMP/ICH/2737/99

ICH Q3B (R2) Impurities in New Drug Products, CPMP/ICH/2738/99;

VICH GL10 Impurities in new veterinary drug substances, CVMP/VICH/837/99 Rev.1

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