Moist-heat autoclaving requirements

Annex 1 Draft 12 (2020-02-20) *vs* Annex 1 in force (2008-11-25)

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Reference documents /1

Reference documents belong to three categories:

- *Regulations (or Rules)*: provide cogent indications for compliance in a national or a super-national area.

  These include: Pharmacopoeias, European Commission Directives, National Laws.

- *Standards*: are produced with the collaboration of various parties (manufacturers, users, standardization and control bodies, *et cetera*) under the aegis of a Standardization Authority, in most cases an international one. Accordingly, they express the "state-of-the-art".

  Typical examples: EN 285, EN-ISO 17665, EN-ISO 11138.

- *Guidelines*: are suggestions for compliance with rules or recommendations according to the point of view of the body that produced them; the compliance is formally free, but Guidelines can carry considerable weight both from a commercial and regulatory point of view, if the issuing body is prestigious.

  The most famous case in our field: PDA TR#1. A very special case: Annex 1 to Eudralex Vol. 4.

Reference documents /2

Meaning and scope of the "Standards"

In spite of being in most cases formally free, the compliance with applicable standards may generate the presumption of compliance *with related Regulations*.

In case of non-compliance with related (or "supporting" or "harmonized") Standards, the inspected Users–at least in Europe or manufacturing *for* Europe or for re-exporting form Europe–are expected to demonstrate that *the applicable Regulations are respected by other means*. In fact, any non-compliance with *"Should"* requirements of EN/ISO Standards, will demand for a thorough demonstration that the different solution adopted is *"at least equivalent to the good manufacturing practice standards laid down by the Community" (see Art 4.2 of Directive 2003/94/EC*. The formulation of this concept in Clause 2.2 of Draft Version 12 for the future new Annex 1 is: *"Where alternative approaches are used, these should be supported by appropriate rationales and risk assessment and should meet the intent of this Annex"*.

Compliance with Standards may also be made mandatory by competent Authorities and/or be the object of commercial requirements.

Reference documents /3

The concept of "Harmonised Standard"

"Devices that are in conformity with the relevant harmonised standards, or the relevant parts of those standards, the references of which have been published in the Official Journal of the European Union, shall be presumed to be in conformity with the requirements of this Regulation covered by those standards or parts thereof."
[ER 2017/745, Art. 8.1]

The official definition of harmonised standard is in turn:

"A harmonised standard is a European standard developed by a recognised European Standards Organisation: CEN, CENELEC, or ETSI. It is created following a request from the European Commission to one of these organisations. Manufacturers, other economic operators, or conformity assessment bodies can use harmonised standards to demonstrate that products, services, or processes comply with relevant EU legislation". [https://ec.europa.eu/growth/single-market/european-standards/harmonised-standards_en#:~:text=A%20harmonised%20standard%20is%20a,to%20one%20of%20these%20organisations.]

The basic pharmaceutical rules in Europe

European Pharmacopoeia (official in 37 Countries)

European Commission Directives (after conversion to national laws)

EudraLex

10 Volumes with several Annexes, containing *"The rules governing medicinal products in the European Union"*.
EudraLex is a *system of Rules*, thanks to the various Directives, including 2003/94/EC "laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use".

The body of Eudralex is compiled in Volume 1 (human) and Volume 5 (veterinary) of the publication. The basic legislation is supported by a series of Guidelines that are published in the other volumes.
Volume 4 contains a "Guidance for the interpretation of the principles and guidelines of good manufacturing practices for human and veterinary use laid down in Commission Directives 2003/94/EC and 91/412/EEC respectively". In short, this Volume is often referred to as "GMPs".

Freely downloadable from Internet.

Annexes to EudraLex Vol. 4

- **Annex 1 to EudraLex Volume 4** deals with the “Manufacture of Sterile Medicinal Products”, formally as “guidance” to a “guideline”

  Version in force on June 29, 2022 issued early in 2008 and amended on November 25, 2008, hereinafter “Annex 1 2008”).

- Other very important Annexes to EudraLex Volume 4 are:

  No. 11 (“Computerised Systems”),

  No. 15 (“Qualification and Validation”),

  No. 17 (“Parametric release”).

Revision of Annex 1 to EudraLex Vol. 4

A first “targeted consultation” on a Revision draft was conducted under the aegis of European Commission from December 20, 2017, to 20 March 20, 2018. On February 20, 2020, the present Draft Version 12 was submitted to a second “targeted consultation”, subsequently extended until July 20, 2020.

Draft Version 12 eliminates “Medicinal” from the title (but also Annex 1 2008 regards “Sterile Products” in general).

It might be expected that before the final approval the text of Draft Version 12 will undergo remarkable changes, due both to the comments received, and, hopefully, to a more synthetical character of the final document. At present, it is still difficult to say when new Annex 1 will be ready and put into force.

“Must” and “Should” in Annex 1

“Must” is a word used very seldom already in Annex 1 2008 (nine times only), i.e. *only to state some rules not yet stated somewhere else*. Even if EudraLex Vol. 4, and its Annexes, are a law in the EU Countries, requirements therein are addressed mostly with “should”.

Draft Version 12 further reduces the use of “Must”, to one single regulatory case: “manufacture of sterile products **must** strictly follow carefully established and validated methods of manufacture and control”.

Despite this, any non-compliance with “Shoulds” of Eudralex Vol. 4 and its Annexes, as well with “supporting” EN/ISO Standards, will demand for a thorough demonstration that the different solution adopted is, as already remembered, “at least equivalent to the good manufacturing practice standards laid down by the Community” and “should be supported by appropriate rationales and risk assessment and should meet the intent of this Annex”.

Annex 1 2008 vs Draft version 12 /1

General criteria

"Principle: Sole reliance for sterility or other quality aspects must not be placed on any terminal process or finished product test".

— Confirmed (with *should*) in Draft Version 12

"Note: This guidance does not lay down detailed methods … Reference should be made to other documents such as the EN/ISO standards."

— This sentence is no longer present in Draft Version 12.

The above "Principle" expresses the concept that sterilization is a "Special Process", i.e., that the good result of a sterilization process cannot be demonstrated by final inspection.

Annex 1 2008 vs Draft version 12 /2

QRM: A major change in the general formulation of the test is relevant to the relatively new approach of Quality Risk Management (QRM), the principles thereof are frequently invoked in Draft Version 12, according to the general new statement in Clause 2.2:

"Process, equipment, facilities and manufacturing activities should be managed in accordance with QRM principles, to provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. ***Where alternative approaches are used, these should be supported by appropriate rationales and risk assessment and should meet the intent of this Annex.*** *QRM priorities should include good design of the facility, equipment and process in the first instance, then implementation of well-designed procedures, with monitoring systems as the final element that demonstrate that the design and procedures have been correctly implemented and continue to perform in line with expectations. Exclusively monitoring or testing does not give assurance of sterility".*

Annex 1 2008 vs Draft version 12 /3

Other previously non used concepts recur often in Draft Version 12, such as:

- **CCS** = Contamination Control Strategy,
- **CAPA** = Corrective and Preventive Actions,
- **PQS** = Pharmaceutical Quality System.

A very important statement is the last one of Clause 3.1:

"the PQS for sterile product manufacture should also ensure that:

i. – vi. …

vii. Persons responsible for the quality release of sterile products have appropriate access to manufacturing and quality information and possess adequate knowledge and experience in the manufacture of sterile products and their critical quality attributes. This is in order to allow such persons to ascertain that the sterile products have been manufactured in accordance with the registered specifications and are of the required quality".

Annex 1 2008 vs Draft version 12 /4

Pyrogens: The very first sentence in Annex 1 2008 states:

"The manufacture of sterile products is subject to special requirements in order to minimize risks of *microbiological contamination, and of particulate and pyrogen contamination*".

By this wording, a slightly greater attention is intended to be paid to microbiological contamination than to other sources of impureness.

In Draft version 12 the Principle begins with a very similar, yet non-identical sentence. Clause 2.1 states:

"The manufacture of sterile products is subject to special requirements in order to minimize risks of *microbial, particulate and pyrogen contamination*".

The new wording puts these three types of contamination on the same level. Draft Version 12 shows throughout an increased attention to all potential sources of contamination, as in Clause 2.5: "microbial and cellular debris (e.g. pyrogen and endotoxins)", thus bringing a major attention to the overall pureness of the product.

Annex 1 2008 vs Draft version 12 /5

Coherently with the above attention, Draft Version 12 Chapter no. 4 (*Premises*) is almost twice long as, and much more detailed than the corresponding parts in Annex 1 2008.

Chapters no. 5 (*Equipment*), no. 6 (*Utilities*), no. 7 (*Personnel*) and the part of no. 8 (*Production and Sterile Technologies*) dealing with Aseptic preparation and processing are also undergoing a remarkable amplification and revision.

All these chapters photograph the mid-high level of the present “state-of-the-art”. For this reason, these Chapters are the most likely ones to be modified before the final text of new Annex 1 is issued.

Bioburden /what it is

Bioburden is defined by Glossary in Draft Version 12 as "The total number of microorganisms associated with a specific item such as personnel, manufacturing environments (air and surfaces), equipment, product packaging, raw materials (including water), in-process materials or finished products".

No doubt that this draft definition is unsatisfactory, because it neglects the very microbiological characteristics of the microorganism.

I suggest to understand under the word bioburden *the combination of these two elements (number and resistance). In most cases, both "should" be monitored before sterilization for a sound design, validation and routine evaluation of the sterilization process.*

Bioburden /Texts

N.B.: Annex 1 2008 in black; Draft Version 12 in blue; unchanged or almost unchanged parts in green.

Clause 80: The bioburden should be monitored before sterilisation. There should be working limits on contamination immediately before sterilisation, which are related to the efficiency of the method to be used. Bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilised products. Where overkill sterilisation parameters are set for terminally sterilised products, bioburden might be monitored only at suitable scheduled intervals. For parametric release systems, bioburden assay should be performed on each batch and considered as an in-process test. Where appropriate the level of endotoxins should be monitored. All solutions, in particular large volume infusion fluids, should be passed through a micro-organism-retaining filter, if possible sited immediately before filling.

Clauses 10.3 and 10.4: The bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilized products and the results considered as part of the final batch review. There should be defined limits for bioburden immediately before the sterilizing filter or the terminal sterilization process, which are related to the efficiency of the method to be used. Samples should be taken to be representative of the worst case scenario (e.g. at the end of hold time). Where overkill sterilization parameters are set for terminally sterilized products, bioburden should be monitored at suitable scheduled intervals. (10.3)

A pre-sterilization bioburden monitoring program for the product and components should be developed to support parametric release. The bioburden should be performed for each batch. The sampling locations of filled units before sterilization should be based on a worst case scenario and be representative of the batch. Any organisms found during bioburden testing should be identified and their impact on the effectiveness of the sterilizing process determined. Where appropriate, the level of pyrogen (endotoxins) should be monitored. (10.4)

Bioburden /Comment

Draft Version 12 emphasizes the importance of bioburden assay ("the results [should be] considered as part of the final batch review") and the representativeness of the samples taken from the batch, with a wording very similar to that for sterility tests (see below, Clause 10.6 under "Quality control"). This stresses the importance of the initial condition of the sterilization process.

New Clause 10.4 for the case of parametric release includes the components among the items to be assayed for bioburden and precisely explains the present recommendation of "bioburden assay as in-process test". This way, there is no doubt, now, that the monitoring of the pyrogen level may be required only for the parametric release.

Sterilization in general /Texts

Clause 83 to 85: All sterilisation processes should be validated. Particular attention should be given when the adopted sterilisation method is not described in the current edition of the European Pharmacopoeia, or when it is used for a product which is not a simple aqueous or oily solution. Where possible, heat sterilisation is the method of choice. In any case, the sterilisation process must be in accordance with the marketing and manufacturing authorisations. (83)

Before any sterilisation process is adopted its suitability for the product and its efficacy in achieving the desired sterilising conditions in all parts of each type of load to be processed should be demonstrated by physical measurements and by biological indicators where appropriate. The validity of the process should be verified at scheduled intervals, at least annually, and whenever significant modifications have been made to the equipment. Records should be kept of the results. (84)

For effective sterilisation the whole of the material must be subjected to the required treatment and the process should be designed to ensure that this is achieved. (85)

Clauses 8.33 to 8.36, and 8.38: Where possible, finished product should be terminally sterilized, using a validated and controlled sterilization process, as this provides a greater assurance of sterility than a validated and controlled sterile filtration process and/or aseptic processing. Where it is not possible for a product to undergo terminal sterilization, consideration should be given to using terminal bioburden reduction steps, such as heat treatments (e.g. pasteurization), combined with aseptic process to give improved sterility assurance. (8.33)

The selection, design and location of the equipment and cycle/programme used for sterilization should be based on scientific principles and data which demonstrate repeatability and reliability of the sterilization process. Critical parameters should be defined, controlled, monitored and recorded. (8.34)

All sterilization processes should be validated. Validation studies should take into account the product composition, storage conditions and maximum time between the start of the preparation of a product or material to be sterilized and its sterilization. Before any sterilization process is adopted, its suitability for the product and equipment, and its efficacy in consistently achieving the desired sterilizing conditions in all parts of each type of load to be processed should be validated notably by physical measurements and where appropriate by biological indicators (BI). For effective sterilization, the whole of the product, and surfaces of equipment and components should be subject to the required treatment and the process should be designed to ensure that this is achieved. (8.35)

Particular attention should be given when the adopted sterilization method is not described in the current edition of the Pharmacopoeia, or when it is used for a product which is not a simple aqueous solution. Where possible, heat sterilization is the method of choice. (8.36)

The validity of the sterilizing process should be reviewed and verified at scheduled intervals based on risk. Heat sterilization cycles should be revalidated with a minimum frequency of at least annually. (8.38)

Sterilization in general /Comment

Draft Version 12 further strengthens by the new Clause 8.33 the assessment in the European Pharmacopoeia, that terminal sterilization by heat is the method of choice to produce sterile products rather than filtration and aseptic process. It includes oily solutions in "difficult" products, emphasizes the concept of consistency and summarizes the extent of validation.

Another meaningful change: Draft Version 12 bases the scheduling of revalidation "on risk" (8.38); Annex 1 2008 bases it on "performance history" and requires revalidation whenever "any significant change is made on the process or equipment" (Clause 82).

Loading patterns

Clause 86: Validated loading patterns should be established for all sterilisation processes.

Clause 8.37: Validated loading patterns should be established for all sterilization processes and should be subject to periodic revalidation. Maximum and minimum loads should also be considered as part of the overall load validation strategy.

Draft Version 12 adds the "shoulds" for periodic revalidation of the loading patterns and regards "minimum load" as object of independent validation.

Loading patterns: a useful explanation makes them easier to validate and sterilization more successful

After the operational qualification and prior to beginning the performance qualification, load types and patterns need to be determined and documented. The following considerations should be given to sterilization effectiveness and production efficiency.

- *Load items should not come into contact with the interior surfaces of the chamber.*
- *Contact between flat surfaces of metal boxes and trays may be minimized by use of racks with perforated, and if necessary, adjustable shelving.*
- *Well-defined item orientation to facilitate air removal, condensate drainage and steam penetration (e.g., buckets should be sterilized upside down) should be documented and only authorized orientations should be used.*
- *Largest mass items should be placed on the lower shelves of the sterilizer to minimize wetting by condensate.*
- *An important consideration for porous/hard goods loads is control over the number of articles in the sterilizer. In the event the load size is expected to vary, minimum and maximum loads should be identified. A sound bracketing approach to qualifying intermediate loads should include the most-difficult-to-sterilize load items.*
- *Variable loading patterns may be used; however, additional qualifications studies should be performed to demonstrate load position does not affect sterilization efficacy.*
- *Loading instructions should be documented and readily available for operator reference.*

(PDA Technical Report no. 1, Clause 4.4.1.3)

Routine and Deviation /Texts

Clauses 8.39 and 8.40: Routine operating parameters should be established and adhered to for all sterilization processes, e.g. physical parameters and loading patterns. (8.39)

There should be mechanisms in place to detect a sterilization cycle that does not conform to the validated parameters. Any failed sterilization or sterilization that deviated from the validated process (e.g. have longer or shorter phases such as heating cycles) should be investigated. (8.40)

Routine and Deviation /Comment

New Clauses 8.39 and 8.40 of Draft Version 12 correspond to practices which have been common and widespread already for tens of years in Europe.

Clause 8.39 makes clearer and absolute the primary role of physical parameters for evaluating the efficacy of a sterilization process. Annex 1 2008 expresses this sparsely, e.g. by Clause 91 (see below, under "Biological indicators").

The first sentence of Clause 8.40 summarizes the concepts expressed in Paragraph 7.2 "Fault indication system" of the European Standard EN 285:2015 relevant to tests and requirements for "large steam sterilizers". This sentence is thus targeted to the design (and validation, indeed) of the control and alarm system of sterilizers.

The second sentence of Clause 8.40 is relevant to quality assurance practices and is targeted to organizational aspects in manufacturing sterile products.

Biological Indicators /what they are

Biological Indicators (BIs) are defined by Glossary in Draft Version 12 as "A population of microorganisms inoculated onto a suitable medium (e.g. solution, container or closure) and placed within a sterilizer or load or room locations to determine the sterilization or disinfection cycle efficacy of a physical or chemical process. The challenge microorganism is selected and validated based upon its resistance to the given process. Incoming lot D value, microbiological count and purity define the quality of the BI".

In PDA TR#1, "Biological Indicator Challenge System (BI)" is defined as "A test system containing viable microorganisms of a pure, specified strain providing a defined resistance to a specified sterilization process".

Biological Indicators /Texts

Clauses 87 and 91: Biological indicators should be considered as an additional method for monitoring the sterilisation. They should be stored and used according to the manufacturer's instructions, and their quality checked by positive controls. If biological indicators are used, strict precautions should be taken to avoid transferring microbial contamination from them. (87)

Chemical or biological indicators may also be used, but should not take the place of physical measurements. (91)

Clauses 8.41 and 8.42: Suitable BIs placed at appropriate locations may be considered as an additional method to support the validation of the sterilization process. BIs should be stored and used according to the manufacturer's instructions. Where BIs are used to support validation and/or to monitor a sterilization process (e.g. for ethylene oxide), positive controls should be tested for each sterilization cycle. If BIs are used, strict precautions should be taken to avoid transferring microbial contamination to the manufacturing or other testing processes. BI results in isolation do not give assurance of sterilization and should not be used to override other critical parameters and process design elements. (8.41)

The reliability of BIs is important. Suppliers should be qualified and transportation and storage conditions should be controlled in order that BI quality is not compromised. Prior to use of a new batch/lot of BIs, the population and identity of the indicator organism of the batch/lot should be verified. For other critical parameters, e.g. D-value, Z- value, the batch certificate provided by the qualified supplier can normally be used. (8.42)

Biological Indicators /Comment

Draft Version 12 confirms the so called "European" approach to the use of BIs by making it clearer and more specific. Even when BI results are necessary, e.g. due to the configuration of the load, their use by itself "do not give assurance of sterilization" and the conformity to validated physical parameters "should" not be overridden.

It also replaces the words "for monitoring the sterilization" of Annex 1 2008 with "to support the validation and/or to monitor a sterilization process". Although BIs have been used in the validation exercise, this change on the text clarifies that the mandatory use of BIs in moist-heat sterilization routine is not within the scope of the revision. Gas sterilization is another world.

Draft Version 12 draws attention on the actual reliability of BIs *(positive controls are foreseen by Annex 1 2008 as well) but does not demand that the final user directly verifies their properties.*

Differentiating products

Clause 88: There should be a clear means of differentiating products which have not been sterilised from those which have. Each basket, tray or other carrier of products or components should be clearly labelled with the material name, its batch number and an indication of whether or not it has been sterilised. Indicators such as autoclave tape may be used, where appropriate, to indicate whether or not a batch (or sub-batch) has passed through a sterilisation process, but they do not give a reliable indication that the lot is, in fact, sterile.

Clause 8.43: There should be a clear means of differentiating products, equipment and components, which have not been subjected to the sterilization process from those which have. Containers used to carry products such as baskets or trays, items of equipment and/or components should be clearly labelled (or electronically tracked) with the material name, product batch number and an indication of whether or not it has been sterilized. Indicators such as autoclave tape, or irradiation indicators may be used, where appropriate, to indicate whether or not a batch (or sub-batch) has passed through a sterilization process. However, these indicators show only that the sterilization process has occurred, they do not indicate product sterility or achievement of the required sterility assurance level.

Draft Version 12 adds a more specific reference to items of equipment and components, and better explains the concept that having been subject to a sterilization process *is not the same as having been effectively* sterilized.

Sterilization records /Texts

Clause 89, 90 and 94 (parts of this): Sterilisation records should be available for each sterilisation run. They should be approved as part of the batch release procedure. (89)

Each heat sterilisation cycle should be recorded on a time/temperature chart with a sufficiently large scale or by other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and/or recording should have been determined during the validation, and where applicable also checked against a second independent temperature probe located at the same position. (90)

Control instrumentation should normally be independent of monitoring instrumentation and recording charts. Where automated control and monitoring systems are used for these applications they should be validated to ensure that critical process requirements are met. System and cycle faults should be registered by the system and observed by the operator. The reading of the independent temperature indicator should be routinely checked against the chart recorder during the sterilisation period. (94)

Clauses 8.44, 8.49 and 8.50: Sterilization records should be available for each sterilization run. Each cycle should have a unique identifier. They should be reviewed and approved as part of the batch certification procedure. (8.44)

Each heat sterilization cycle should be recorded either electronically or by hardcopy, on equipment with suitable accuracy and precision. Monitoring and recording systems should be independent of the controlling system (e.g. by the use of duplex/double probes). (8.49)

The position of the temperature probes used for controlling and/or recording should be determined during the validation which should include heat distribution and penetration studies and, where applicable, also checked against a second independent temperature probe located at the same position. (8.50)

Sterilization records /Comment

Draft Version 12 adds the "should" for the uniqueness of identification of the batches (a current GMP, indeed) and implicitly states that any release demands for a certification.

It also adds that both heat distribution *(i.e. temperature uniformity) and* heat penetration *(i.e. sufficient duration of the exposure to temperature) are to be included in the validation studies: the recording system should be independent of the monitoring one.*

In their comments, PDA claim that the example in Clause 8.49 ("e.g. by the use of duplex/double probes") could result in "limiting the use of modern or innovative technologies", and that in Clause 8.50 "the positioning of monitoring probes should be known and in place for the validation study, rather than determined as a variable during the study".

Heat Penetration

Clause 92: Sufficient time must be allowed for the whole of the load to reach the required temperature before measurement of the sterilising time-period is commenced. This time must be determined for each type of load to be processed.

Clause 8.51: Sufficient time should be allowed for the whole of the load to reach the required temperature before measurement of the sterilizing time-period starts. For sterilization cycles controlled by using a reference probe within the load, specific consideration should be given to ensuring the load probe temperature is controlled within defined temperature range prior to cycle commencement.

Draft Version 12 deletes the remark that heat penetration time must be determined for each type of load to be processed, as implicitly different for each type — but it also considers the first sentence of Clause 8.45: "Where possible, materials, equipment and components should be sterilized by validated methods appropriate to the specific material".

In addition, it adds the less obvious warning that "the probe within the load", if present, shall not commence the cycle in a too warm condition. This may become critical in cycles for liquids.

Equilibration Time

Annex 1 2008 avoids, and Draft Version 12 uses only once the words "equilibration time", but this represents one of the most common problems in sterilization of porous/hard goods.

According to Glossary of PDA TR#1, equilibration time is "The period that elapses between the attainment of the minimum exposure temperature at the reference measurement point (typically the drain) and the attainment of the sterilization temperature at all points within the load. This period is an indication of the ability to properly remove air and heat the load items; consequently, it is typically only evaluated for heat penetration probes placed in porous/hard good loads".

The same authoritative guideline states (Clause 4.4.1.5): "Extended equilibration times can be indicative of inadequate air removal or heating, even if the desired temperature is eventually achieved. When developing a cycle it is important to take practical precautions to minimize equilibration time."

*This recommendation is not in contradiction with Annex 1 requirement: "*Sufficient time should be allowed for the whole of the load to reach the required temperature before measurement of the sterilizing time-period starts". A too long equilibration time must be avoided as it brings the risk of heating the load by other heat-transfer mechanisms than steam condensation. "Sufficient" does not mean "as extended as you like".

Equilibration Time /Requirements

EN 285:2015 (several clauses):

The requirements for equilibration time not exceeding 30 (or 15) seconds are referred to ***test loads.*** *The requirement on equilibration time duration is part of specification of the sterilizer and has the aim to demonstrate, by mean of the standard test load, that the sterilizer is compliant with the Standard as far as the removal air capacity is concerned.*

The meaning of the upper limit for the equilibration time is apparent:

a) to prevent that the desired temperature is eventually achieved by heat transmission instead of steam penetration;

b) to avoid that the effective exposure time (or holding time) is too much overrated, because the calculation of the exposure time usually begins when the reference measurement point has overtaken the minimum sterilization temperature, (or at the beginning of the plateau period) even if at this moment not all the load has already done the same.

EN ISO 17665-1:2006 (Clause 8.11):

"The SAL attained on and/or within the product during the sterilization process shall

…

c) be defined by demonstrating that during the holding time all parts of the product are exposed to process parameters selected from an official national or regional pharmacopoeia or

d) be deemed to be equal to or to exceed the requirements specified in c), ***provided that the product is assigned to a product family for which a sterilization process is specified and that the equilibration time does not exceed the maximum for products assigned to the same product family****."*

Conclusions: *Equilibration time is a variable parameter which shall be minimized during the cycle development and its allowed maximum shall be included among the acceptance criteria for any actual sterilization process.* ***Anymore, the acceptability of an equilibration time for porous/hard goods exceeding the minimum value required for test loads shall be determined by biological challenge for any load and any loading pattern.***

Avoiding recontamination /Texts

Clauses 93 and 95: After the high temperature phase of a heat sterilisation cycle, precautions should be taken against contamination of a sterilised load during cooling. Any cooling fluid or gas in contact with the product should be sterilised unless it can be shown that any leaking container would not be approved for use. (93)

The items to be sterilized, other than products in sealed containers, should be wrapped in a material which allows removal of air and penetration of steam but which prevents recontamination after sterilization. All parts of the load should be in contact with the sterilizing agent at the required temperature for the required time. (95)

Clause 8.45 (in part): Suitable protection after sterilization should be provided to prevent recontamination

Clauses 8.52 and 8.60: After completion of the high temperature phase of a heat sterilization cycle, precautions should be taken against contamination of a sterilized load during cooling. Any cooling liquid or gas that comes in contact with the product or sterilized material should be sterilized. (8.52)

The items to be sterilized, other than products in sealed containers, should be dry, wrapped in a material which allows removal of air and penetration of steam and prevents recontamination after sterilization. All loaded items should be dry upon removal from the sterilizer. Load dryness should be confirmed by visual inspection as a part of the sterilization process acceptance. (8.60)

Avoiding recontamination /Comment

*Draft Version 12 extends to any sterilized material the precaution previously intended for product only (“*Any cooling liquid or gas that comes in contact with the product or sterilized material should be sterilized*”) but restricts it to the case that the high temperature phase has been completed (i.e. that the sterilization has not been aborted). No exception more is allowed to sterilized media for cooling.*

The new requirement for dryness of items prior to sterilization admits a meaningful exception (see below, Clause 8.61 under “Moist heat sterilization”).

Moist-heat sterilization /1

Clause 8.54: Moist heat sterilization utilises steam or superheated water, typically at lower temperatures and shorter duration than dry heat processes, in order to sterilize a product or article. Moist heat sterilization of hard goods or porous loads is primarily effected by latent heat of condensation of clean steam and the quality of steam is therefore important to provide consistent results. For aqueous liquid-filled containers, energy from moist heat is transferred through conduction and/or convection to the content of the container without direct contact with the autoclave steam. In these cases, time and temperature are the key parameters and steam quality does not have the same impact to the process. Moist heat sterilization processes may be utilized to sterilize or control bioburden (for non sterile applications) of thermally stable materials, articles or products and is the preferred method of sterilization, where possible. Moist heat sterilization can be achieved using steam, (direct or indirect contact), but also includes other systems such as superheated water systems. Superheated systems are typically used for the terminal sterilization of product in flexible containers where the pressure differentials associated with the steam would cause damage to the primary container.

Clause 8.54 of Draft Version 12 describes meaning and purpose of Moist-heat sterilization (more in general, Moist-heat thermal treatment).

Moist-heat sterilization /2 - Texts

Clause 94 (beginning): Both temperature and pressure should be used to monitor the process. Control instrumentation should normally be independent of monitoring instrumentation and recording charts. Where automated control and monitoring systems are used for these applications they should be validated to ensure that critical process requirements are met. System and cycle faults should be registered by the system and observed by the operator. The reading of the independent temperature indicator should be routinely checked against the chart recorder during the sterilisation period. For sterilisers fitted with a drain at the bottom of the chamber, it may also be necessary to record the temperature at this position, throughout the sterilisation period …

Clauses 8.55 to 8.57: For porous cycles (hard goods) time, temperature and pressure should be used to monitor the process. Each item sterilized should be inspected for damage, packaging material integrity and moisture on removal from the autoclave. Any item found not to be fit for purpose should be removed from the manufacturing area and an investigation performed. (8.55)

For autoclaves fitted with a drain at the bottom of the chamber, the temperature should be recorded at this position throughout the sterilization period. For steam in place systems, the temperature should be recorded at condensate drain locations throughout the sterilization period. (8.56)

Validation of porous cycles should include a calculation of equilibration time, exposure time, correlation of pressure and temperature and maximum temperature range during exposure. Validation of fluid cycles should include temperature, time and/or Fo. These critical processing parameters should be subject to defined limits (including appropriate tolerances) and be confirmed as part of the sterilization validation and routine cycle acceptance criteria. (8.57)

Moist-heat sterilization /2 - Comment

Clause 8.55 of Draft Version 12 confirms, with a more precise wording, the importance of monitoring pressure in "porous cycles" and adds the "shoulds" for inspecting the items "on removal from the autoclave" and rejecting them immediately if no longer "fit for purpose".

Clause 8.56 of Draft Version 12 converts to a constant "should" the recording of the temperature at the drain, if present, "throughout the sterilization period", regardless to the moist-heat sterilization method, and applies it also to "steam in place systems", previously not addressed.

Clause 8.57 of Draft Version 12 describes the current "state-of-the-art" for the validation of Moist-heat thermal treatment. It also underlines that equivalent time F_0 *is not intended for replacing exposure time in the case of porous loads, and that validation of "*equilibration time*" doesn't apply to liquid loads.*

Moist-heat sterilization /3

Clause 94 (end): … There should be frequent leak tests on the chamber when a vacuum phase is part of the cycle.

Clauses 8.58: Leak tests on the sterilizing system should be carried out periodically (normally weekly) when a vacuum phase is part of the cycle or the system is returned, post-sterilization, to a pressure lower than the environment surrounding the sterilized system.

Clause 8.58 of Draft Version 12 includes the whole "sterilizing system" (i.e. critical fittings) in leak tests when applicable. It also converts the formerly "frequent" leak tests in "periodical" ones and explains (perhaps unnecessarily) that, from this point of view, there is no difference between vacuum phases prior and after the sterilization period.

Clause 8.59: There should be adequate assurance of air removal prior to and during sterilization when the sterilization process includes air purging (e.g. porous autoclave loads, lyophilizer chambers). For autoclaves, this should include an air removal test cycle (normally performed on a daily basis) or an air detector system. Loads to be sterilized should be designed to support effective air removal and be free draining to prevent the build-up of condensate.

Clause 8.59 of Draft Version 12 describes another current "state-of-the-art" and formalizes that "an air removal test cycle (normally performed on a daily basis)" is intended as equivalent to the presence of "an air detector system". This clause also stresses that design of loads to be sterilized should consider "effective air removal" and condensate drainage: this is a completely new remark

(perhaps suggested by PDA TR#1, Clause 4.4.1.5: "optimize steam exposure to load items").

Moist-heat sterilization /4

For the new Clause 8.60 see above, under "Avoiding recontamination" (slide no. 31).

Clause 8.61: If it is necessary to wet equipment using WFI (e.g. ultrafiltration membrane) prior to the sterilization process, then a risk-based assessment should be carried out to demonstrate the acceptable dryness level that will not impact the sterility of the equipment sterilized and the product sterility assurance level. The hold time between the wetting phase and sterilization should be justified and validated. (8.61)

New Clause 8.61 regards the practice of adding small quantities of suitable water to guarantee Moist-heat condition during the sterilization process and brings the attention to the risks both of insufficient dryness after completion of the process and microbial growth "between the wetting phase and sterilization".

Clause 8.62: Distortion and damage of non-rigid containers that are terminally sterilized, such as containers produced by Blow-Fill-Seal or Form-Fill-Seal technologies, should be prevented by appropriate cycle design and control (for instance setting correct pressure, heating and cooling rates and loading patterns).

New Clause 8.62 of Draft Version 12 formalizes as a "should" the current User's requirement for sterilization of non-rigid containers.

Moist-heat sterilization /5

Clause 8.63: Where steam in place systems are used (e.g. for fixed pipework, vessels and lyophilizer chambers), the system should be appropriately designed and validated to assure all parts of the system are subjected to the required treatment. The system should be monitored for temperature, pressure and time at appropriate locations during routine use to ensure all areas are effectively and reproducibly sterilized. These locations should be demonstrated as being representative of, and correlated with, the slowest to heat locations during initial and routine validation. Once a system has been sterilized by steam in place it should remain integral and held under positive pressure prior to use.

Clause 8.63 of Draft Version 12 describes the current "state-of-the-art" for steaming in place and formalizes that this practice should be validated and monitored according to the same criteria of "porous cycles".

Moist-heat sterilization /6

Clauses 8.64 and 8.65: For systems using superheated water rather than steam, as the sterilizing agent, the heated water should consistently reach all of the required contact points. Initial qualification studies should include temperature mapping of the entire load. There should be routine checks on the equipment to ensure that nozzles (where the water is introduced) are not blocked and drains remain free from debris. (8.64)

For the qualification of superheated systems it should be demonstrated that all parts of the load meet the minimum required temperature and that routine monitoring probes are located in the worst case positions identified during the qualification process. (8.65)

Clauses 8.64 and 8.65 of Draft Version 12 describe the current "state-of-the-art" for superheated water sterilizers, thus demanding for effective distribution of the heating medium on the load, i.e. on all "the required contact points" of it, and for actual attainment of the "minimum required temperature". No reference is made to the time as critical parameter, as in this case it can be replaced by the equivalent time F_0 (see Clause 8.57 here above). For the reliability of the temperature measured by "in product" probes, see also Clause 8.51 under "Heat penetration".

Steam used as a direct sterilizing agent /Text

Clause 96: Care should be taken to ensure that steam used for sterilisation is of suitable quality and does not contain additives at a level which could cause contamination of product or equipment.

Clauses 6.16 and 6.17: Feed water to a pure steam (clean steam) generator should be appropriately purified. Pure steam generators should be designed, qualified and operated in a manner to ensure that the quality of steam produced meets defined chemical and endotoxin levels. (6.16)

Steam used as a direct sterilizing agent should be of suitable quality and should not contain additives at a level which could cause contamination of product or equipment. For a pure steam generator supplying pure steam used for the direct sterilization of materials or product-contact surfaces (e.g. porous hard-good autoclave loads), steam condensate should meet the current monograph for WFI of the relevant Pharmacopeia. A suitable sampling schedule should be in place to ensure that representative pure steam samples are obtained for analysis on a regular basis. Other aspects of the quality of pure steam used for sterilization should be assessed periodically against validated parameters. These parameters should include the following: non-condensable gases, dryness value (dryness fraction) and superheat. (6.17)

Steam used as a direct sterilizing agent /Comment

Draft Version 12 turns manufacturers' attention to the production of steam to be used as direct *sterilizing agent (sometimes called "contact steam") and the evaluation of it. The new clauses implicitly allow for industrial steam as indirect heating agent, e.g. in superheated water sterilization processes, and fix the pureness of steam condensate as quality criterion for the steam. The concept of "suitable quality" is explicated by remembering the three most common tests for steam quality referred to in the widely used Technical Standard EN 285:2015. In fact, the updating is a photography of the current GMP in Pharma industry.*

The new clauses on "contact steam" are part of Chapter 6, titled Utilities*, that also deals with requirements for* Water systems, Gases and vacuum systems, *and* Heating and cooling and hydraulic systems. *These requirements refer to the "current Pharmacopoeia" where appropriate (WFI, gas quality) and once again photograph current GMP, both for design and construction criteria and ongoing monitoring of these systems.*

Quality control /1

Clause 125: The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured. The test should be validated for the product(s) concerned.

Clause 10.5: The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured. It cannot be used to assure sterility of a product that does not meet its design, procedural or qualification parameters. The test should be validated for the product concerned.

Clause 10.5 of Draft Version 12 clearly explains that a product finally tested as sterile cannot be regarded as having been correctly sterilized according to the designed and qualified process, just as biological indicators "in isolation do not give assurance of sterilization and should not be used to override other critical parameters and process design elements" (see above, Clause 8.41 under "Biological indicators").

For the revision of Clause 126 see below, under "Parametric release".

Quality control /2

Clause 127: Samples taken for sterility testing should be representative of the whole of the batch, but should in particular include samples taken from parts of the batch considered to be most at risk of contamination, e.g.:

a. for products which have been filled aseptically, samples should include containers filled at the beginning and end of the batch and after any significant intervention,

b. for products which have been heat sterilised in their final containers, consideration should be given to taking samples from the potentially coolest part of the load.

Clause 10.6: The sterility test should be performed under aseptic conditions. Samples taken for sterility testing should be representative of the whole of the batch but should in particular include samples taken from parts of the batch considered to be most at risk of contamination, for example:

Quality control /3

i. [Relevant to products which have been filled aseptically]

ii. For products which have been heat sterilized in their final containers, samples taken should be representative of the worst case locations (e.g. the potentially coolest or slowest to heat part of each load).

iii. For products that are lyophilized, samples taken from different lyophilization loads.

Note: Where the manufacturing process results in sub-batches (e.g. for terminally sterilized products) then sterility samples from each sub-batch should be taken and a sterility test for each sub-batch performed. Consideration should also be given to performing separate testing for other finished product tests.

Clause 10.6 of Draft Version 12 extends the examples to the case of lyophilization and strengthens the concept of the choice of samples as representative of the whole. The "should" relevant to the aseptic conditions for the sterility test is new, but it corresponds to an already widespread practice.

Quality control /4

Clause 10.7: For some products it may not be possible to perform a sterility test prior to release because the shelf life of the product is too short to allow completion of a sterility test. In these cases, the CCS *[Contamination Control Strategy]* should clearly capture the identified risks, the additional considerations of design of the process and additional monitoring required to mitigate the identified risks. (10.7)

Clause 10.7 of Draft Version 12 introduces a meaningful case of exemption from final Sterility tests, other than for parametric release. The clause emphasizes the role of risk analysis in the approach to production and acceptance of sterile products.

Quality control /5

Clauses 10.8 to 10.11: Any process (e.g. Vaporized Hydrogen Peroxide or VH202, Ultra Violet) used to decontaminate the external surfaces of sterility samples prior to testing should not negatively impact the sensitivity of the test method. (10.8)

Media used for environmental monitoring and APS *[Aseptic Process Simulation]* should be tested for its growth promotion capability, in accordance with a formal written program. (10.9)

Environmental monitoring data and trend data generated for classified areas should be reviewed as part of product batch certification. A written plan should be available that describes the actions to be taken when data from environmental monitoring are found out of trend or exceeding the established limits. For products with short shelf life, the environmental data for the time of manufacture may not be available; in these cases, the certification should include a review of the most recent available data. Manufacturers of these products should consider the use of rapid monitoring systems. (10.10)

Where rapid and automated microbial methods are used for general manufacturing purposes, these methods should be validated for the product(s) or processes concerned. (10.11)

Clauses 10.8 to 10.11 of Draft Version 12 regard the organization of the Quality control. As such they have an indirect yet meaningful impact on sterilization GMPs.

Parametric release /What it is

Concept (Annex 17 to EudraLex Vol. 4, Principle): "In specific circumstances, where authorised, based on product knowledge and process understanding, information collected during the manufacturing process can be used instead of end-product testing for batch release".

Definition 1 (Annex 17, Clause 4.1): "…the release of a batch of terminally sterilised product based on a review of critical process control parameters rather than requiring an end-product testing for sterility".

Definition 2 (Annex 17, Glossary): "One form of RTRT *[Real Time Release Testing]*. Parametric release for terminally sterilised product is based on the review of documentation on process monitoring (e.g. temperature, pressure, time for terminal sterilization) rather than the testing of a sample for a specific attribute".

Justification (Annex 17, Clause 4.2): "In contrast *[with "end-product testing for sterility"]*, data derived from in-process controls (e.g. pre-sterilization product bioburden or environmental monitoring) and by monitoring relevant sterilization parameters can provide more accurate and relevant information to support sterility assurance of the product".

Limitation (Annex 17, Clause 4.3): "Parametric release can only be applied to products sterilised in their final container using either moist heat, dry heat or ionising radiation (dosimetric release), according to European Pharmacopoeial requirements".

Parametric release

See also "Bioburden", Clause 80 and Clauses 10.3. to 10.4.

Clause 126: In those cases where parametric release has been authorised, special attention should be paid to the validation and the monitoring of the entire manufacturing process.

Clause 8.53: In those cases where parametric release has been authorized, a robust system should be applied to the product lifecycle validation and the routine monitoring of the manufacturing process. This system should be periodically reviewed. Further guidance regarding parametric release is provided in Annex 17.

In Annex 1 2008, Parametric release was only addressed as a particular case for enhanced bioburden assay and monitoring of the manufacturing process. Draft Version 12 converts the "special attention to be paid to the validation and the monitoring of the entire manufacturing process" into a "robust system to be applied to product lifecycle validation and the routine monitoring of the manufacturing process".

Conclusions

As far as the sterilization proceedings are concerned, Draft Version 12 expresses the demand to ameliorate the present average level of safety and quality in the manufacture of the sterile products by means of a standardization at the state-of-the-art.

Good level producers do not have to expect but minor changes in their manufacturing practice.

Thank you

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