Moist-heat autoclaving requirements in New Annex 1 (2022-08-22) *vs* Old Annex 1 (2008-11-25)

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Reference documents / 1

Reference documents are traditionally divided in 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 are 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.

  Special cases: Annex 1 to EudraLex Vol. 4 and EMA Sterilization Guideline 2019.

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 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)*. This concept is clearly expressed also in Clause 2.2 of Annex 1 2022 *(see here below, Slide no. 12)*.

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”

The official definition of harmonised standard :

“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 concept may also be expressed in a slightly different form:

“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 basic pharmaceutical rules in Europe

European Pharmacopoeia (official in almost forty 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 and now most important 2017/1752/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".

EC Directives and EudraLex are 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 "technical guidance on the principles and guidelines of good manufacturing practice"

The version still in force today, has been issued early in 20-08 and amended on November 25, 20-08 (hereinafter "Annex 1 20-08"). The new version will come in force on August 25, 20-23 (hereinafter "Annex 1 20-22")

Other 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, Draft Version 12 was submitted to a second “targeted consultation”, subsequently extended until July 20, 2020.

Draft Version 12 had eliminated “Medicinal” from the title (but also Annex 1 2008 regards “Sterile Products” in general). 2022 final version has reintegrated it.

Revision of Annex 1 to EudraLex Vol. 4

"The GMP/GDP* Inspectors Working Group and the PIC/S* Committee jointly recommend that the current version of annex 1, on the manufacture of sterile medicinal products, is revised **to reflect changes in regulatory and manufacturing environments**. The new guideline should **clarify** how manufacturers can take **advantage of new possibilities deriving from the application of an enhanced process understanding** by using innovative tools as described in the ICH Q9 and Q10 guidelines.

The revision of Annex 1 should also **take into account related changes in other GMP chapters and annexes** as well as in other regulatory documents. The revised guideline will seek to **remove ambiguity and inconsistencies** and will **take account of advances in technologies**."

[Annex 1 2022, Reason for changes]

* Good Distribution Practice
** Pharmaceutical Inspection Convention and cooperation Scheme

Revision of Annex 1 to EudraLex Vol. 4

First issue in 1971

Several targeted updates

Full review (started 2015)

- QRM Principles;
- New sections;
- Restructured to give more logical flow;
- Added details to a number of the previous sections to provide further clarity

Publication of the final and definitive version August 22, 2022

In force on August 25, 2023 *

* August 25, 2024, for point 8.123 related to *Lyophilizer sterilization and barrier technology.*

"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".

Annex 1 2022 eliminates completely the use of "Must" as regulatory expression.

Despite this, any non-compliance with "Shoulds" of Eudralex Vol. 4 and its Annexes, as well with "supporting" EN/ISO Standards, will demand 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".

Directory 2017/1752/EC is the juridical base that makes Annex 1 2022 mandatory-in-practice.

Annex 1 2022 vs Annex 1 2008

N.B. Annex 1 2008 in black; Annex 1 2022 in blue; unchanged or almost unchanged parts in green.

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 Annex 1 2022

"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 Annex 1 2022.

The above "Principle" expresses the fact that the good result of a sterilization process cannot be demonstrated by final inspection without making the product unusable for its intended purpose. In the past this was expressed by saying that sterilization is a "Special Process".

In this and the next slides, the sentences passed unchanged from Annex 1 2008 into Annex 1 2022 are written in green. New sentences and new wordings are written in blue: they are a very large part of Annex 1 2022.

Annex 1 2022 vs Annex 1 2008 / 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, risk assessment and mitigation, and should meet the intent of this Annex.

In the first instance, QRM priorities should include appropriate design of the facility, equipment and processes, followed by the implementation of well-designed procedures, and finally application of monitoring systems as the element that demonstrates that the design and procedures have been correctly implemented and continue to perform in line with expectations. Monitoring or testing alone does not give assurance of sterility".

Annex 1 2022 vs Annex 1 2008 / 3

Other previously not yet used concepts recur often in Annex 1 2022, 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 certification/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 the associated critical quality attributes. This is in order to allow such persons to determine if the sterile products have been manufactured in accordance with the registered specifications and approved process and are of the required quality".

Annex 1 2022 vs Annex 1 2008 / 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 Annex 1 2022 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 endotoxin/pyrogen contamination*".

The new wording puts these three types of contamination on the same level. Annex 1 2022 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) as well as particulate (e.g. glass and other visible and sub-visible particles)", thus bringing a major attention to the overall pureness of the product.

Annex 1 2022 vs Annex 1 2008 / 5

Coherently with the above attention, Annex 1 2022 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 have also undergone a remarkable amplification and revision.

All these chapters photograph the mid-high level of the present “state-of-the-art”.

Sterilization in Annex 1 2022

Index of the matter

Clauses 8.34 to 8.49 = “Sterilisation”

- applicable to all methods of sterilization

Clauses 8.50 to 8.54 = “Sterilisation by heat”

- applicable only to moist-heat and dry-heat sterilization

Clauses 8.55 to 8.65 = “Moist heat sterilisation”

- applicable only to moist-heat sterilization

Clauses 8.66 to 8.139: relevant to

- sterilization by dry-heat, radiation, ethylene oxide and filtration
- lyophilization
- the preparation and handling of FFS, BFS, closed systems and SUS

Bioburden / what it is

Bioburden is defined by Glossary in Annex 1 2022 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".

In my opinion, this definition is unsatisfactory, because it neglects the specific microbiological characteristics of the microorganisms.

I recommend to understand under the word bioburden *the combination of the 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 / Annex 1 2008

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.

Bioburden / Annex 1 2022

Clause 10.3: 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 sterilising grade 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 sterilisation parameters are set for terminally sterilised products, bioburden should be monitored at suitable scheduled intervals.

Clause 10.4: For products authorised for parametric release, a supporting pre-sterilisation bioburden monitoring programme for the filled product prior to initiating the sterilisation cycle should be developed and the bioburden assay should be performed for each batch. The sampling locations of filled units before sterilisation 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 endotoxin/pyrogen should be monitored.

Bioburden / Comment

Annex 1 2022 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 like 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 de facto *the components among the items to be assayed for bioburden and precisely explains the old recommendation of "bioburden assay as in-process test".*

It is not definitely clear, however, whether the monitoring of the pyrogen level may now be required only *for the parametric release.*

Sterilization in general / Annex 1 2022

Two new introductory clauses

Clause 8.34: Where possible, finished product should be terminally sterilised, using a validated and controlled sterilisation 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 sterilisation, consideration should be given to using post-aseptic process terminal heat treatment, combined with aseptic process to give improved sterility assurance.

Clause 8.35: The selection, design and location of the equipment and cycle/programme used for sterilisation should be based on scientific principles and data which demonstrate repeatability and reliability of the sterilisation process. All parameters should be defined, and where critical, these should be controlled, monitored and recorded.

Sterilization in general / Comment

Annex 1 2022 further strengthens by the new Clause 8.34 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 also emphasizes the concept of consistency and summarizes the extent of validation.

A scientific base for choices, repeatability, reliability and a thorough definition of process parameters may be read as an application of the QRM approach (new Clause 8.35).

Validation in general / Annex 1 2008

Clause 83: 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.

Clause 84: 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. (…)

Clause 85: 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.

Validation in general / Annex 1 2022

Clause 8.36: All sterilisation 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 sterilised and its sterilisation. Before any sterilisation process is adopted, its suitability for the product and equipment, and its efficacy in consistently achieving the desired sterilising 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.

Clause 8.37: Particular attention should be given when the adopted sterilisation 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.

Revalidation / 2022 *vs* 2008

2008

Clause 84: (…) 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.

2022

Clause 8.39: 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 for load patterns that are considered worst case. Other load patterns should be validated at a frequency justified in the CCS.

Validation & Revalidation / Comment

The requirement added in Clause 8.36 of Annex 1 2002 is concerned with the preparation, preservation and conditioning of items or products to be sterilized, as typical of the new document.

The same clause details the meaning of "material" and eliminates the requirement for complying with "marketing and manufacturing authorisations" because they are the specific object of other rules.

In Clause 8.37, oily solution are no longer regarded as similar to aqueous one (and this is very sound).

Another meaningful change: Annex 1 2008 was basing the scheduling of revalidation on "performance history" and requiring revalidation whenever "any significant change is made on the process or equipment" (Clause 82). Annex 1 2022 bases it "on risk" (Clause 8.39).

Loading patterns: some useful explanations

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]

Loading patterns / 2022 *vs* 2008

2008

Clause 85 : Validated loading patterns should be established for all sterilisation processes.

2022

Clause 8.38 : 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.

Annex 1 2022 adds the "shoulds" for periodic revalidation of the loading patterns and regards "minimum load" as object of independent validation.

Routine and Deviation / Annex 1 2022

Two new clauses

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

Clause 8.41: 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.

Routine and Deviation / Comment

New Clauses 8.40 and 8.41 of Annex 1 2022 correspond to practices which have been common and widespread already for tens of years in Europe.

Clause 8.40 makes clearer and absolute the primary role of physical parameters for evaluating the efficacy of a sterilization process. Annex 1 2008 has expressed this only sparsely, e.g. by Clause 91 (see below, under "Biological indicators").

The first sentence of Clause 8.41 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 8.41 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 Annex 1 2022 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 / Annex 1 2008

Clause 87: 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.

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

Biological Indicators / Annex 1 2022

Clause 8.42: Suitable BIs placed at appropriate locations should 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 sterilisation 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 should not be used to override other critical parameters and process design elements.

Clause 8.43: 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, purity 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.

Biological Indicators / Comment

Annex 1 2022 makes clearer and more specific the approach to the use of BIs.

Even when BI results are necessary, e.g. due to the configuration of the load, 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". This change clarifies that the mandatory use of BIs in moist-heat sterilization routine is not within the scope of the revision, even if BIs have been used in the validation exercise. Gas and vapor sterilization is another world.

Annex 1 2022 draws attention on the actual reliability of BIs *(positive controls were foreseen by Annex 1 2008 as well) but does not demand that the final user directly verifies their thermal properties.*

Differentiating products / Annex 1 2008

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.

Differentiating products / Annex 1 2022

Clause 8.44: 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. Equipment such as baskets or trays used to carry products, other items of equipment and/or components should be clearly labelled (or electronically tracked) with the product name and batch number and an indication of whether or not it has been sterilised. Indicators such as autoclave tape, or irradiation indicators may be used, where appropriate, to indicate whether or not a batch (or sub-batch material, component, equipment) has passed through a sterilisation process. However, these indicators show only that the sterilisation process has occurred, they do not indicate product sterility or achievement of the required sterility assurance level.

Annex 1 2022 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 / 2022 *vs* 2008

2008

Clause 89: Sterilisation records should be available for each sterilisation run. They should be approved as part of the batch release procedure.

2022

Clause 8.45: Sterilization records should be available for each sterilisation run. Each cycle should have a unique identifier. Their conformity should be reviewed and approved as part of the batch certification/release procedure.

Annex 1 2022 adds the "should" for the uniqueness of identification of the batches (a current GMP, indeed) and implicitly states that any release demands a certification.

New Clauses 8.46 (most part of it) to 8.49 of Annex 1 2022 deal with organizational and environmental aspects of manufacturing sterile products.

Avoiding recontamination / Annex 1 2008

Clause 93: 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.

Clause 95: 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.

Avoiding recontamination / Annex 1 2022

Clause 8.46: (…) Suitable protection after sterilization should be provided to prevent recontamination (…)

Clause 8.53: After completion of the high temperature phase of a heat sterilisation cycle, precautions should be taken against contamination of a sterilised load during cooling. Any cooling liquid or gas that comes into contact with the product or sterilised material should be sterilised.

Clause 8.56: The items to be sterilised, other than products in sealed containers, should be dry, packaged in a protective barrier system which allows removal of air and penetration of steam and prevents recontamination after sterilisation. All loaded items should be dry upon removal from the steriliser. Load dryness should be confirmed by visual inspection as a part of the sterilisation process acceptance.

Avoiding recontamination / Comments

Annex 1 2022 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 sterilised material should be sterilised") *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 old exception was in fact unpracticable).*

The new requirement in Clause 8.56 for dryness of items prior to moist-heat sterilization ("to be sterilised") *admits exception only in the case of* "products in sealed containers". *All this clause, with the requirement for dryness "upon removal from the steriliser" is included in the section "Moist heat sterilisation", so that, perhaps paradoxically, a dry-heat process could not be the object of dryness requirements.*

Heat-sterilization records / Annex 1 2008

Clause 90: 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.

Clause 94: (…) 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. (…)

Heat-sterilization records / Annex 1 2022

Clause 8.50: Each heat sterilisation cycle should be recorded either electronically or by hardcopy, using equipment with suitable accuracy and precision. The system should have safeguards and/or redundancy in its control and monitoring instrumentation to detect a cycle not conforming to the validated cycle parameter requirements and abort or fail this cycle (e.g. by the use of duplex/double probes connected to independent control and monitoring systems).

Clause 8.51: The position of the temperature probes used for controlling and/or recording should be determined during the validation and selected based on system design in order to correctly record and represent routine cycle conditions. Validation studies should be designed to demonstrate the suitability of system control and recording probe locations, and should include the verification of the function and location of these probes by the use of an independent monitoring probe located at the same position during validation.

Heat-sterilization records / Comments

Both these clauses are almost completely re-written and formalize a "good validation practice" popular since some tens of years.

The second part of new Clause 8.50 can be read in parallel with the first sentence of Clause 8.41, that summarizes the concept of "Fault indication system": this shall be guaranteed by a "redundancy system" independent of the control system.

New Clause 8.51 states that the meaningfulness of routine measurements of physical parameters shall be preventively validated by independent probes, also for dry-heat sterilization. Locations, and the number indeed, of recording probes are the object of "validation studies", which are expected to be based on rationales.

The basic concepts are:

- the non-conformity of a run shall be detected automatically, and the run aborted or failed
- the recording system shall be independent of the monitoring one
- the position of the routine probes shall have been validated

Heat Penetration / 2022 *vs* 2008

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 8.52: The whole of the load should reach the required temperature before measurement of the sterilising time 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.

Heat Penetration / Comments

Annex 1 2022 deletes the remark that heat penetration time "must" be determined for each type of load to be processed, as the remark is implicit in the new text of Clause 8.52.

In addition, Clause 8.52 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 be critical in monitoring and recording cycles for liquids.

Equilibration Time / Current definition

Annex 1 2008 has avoided, and Annex 1 2022 uses only once the words "equilibration time", but this represents one of the most common issues in the sterilization practice of porous/hard goods.

EN ISO 17665-1:2006:

"3.13
equilibration time
period which elapses between the attainment of the sterilization temperature at the reference measurement point and at all points within the load"

"3.41
reference measurement point
point where the temperature probe used for the operating cycle control is located"

Equilibration Time / PDA interpretation

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 2022 requirement: "*The whole of the load should reach the required temperature before measurement of the sterilising time starts". Too long an equilibration time must be avoided as it brings the risk of heating the load by other heat-transfer mechanisms than steam condensation. "Sufficient time" (as previously written in Annex 1 2008) does not mean "as extended as you like".

Equilibration Time / Requirements I

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 apparently to prevent that the desired temperature is eventually achieved by heat transmission instead of steam penetration.

In addition, an upper limit for equilibration time prevents the effective exposure time (or holding time) from the risk of being too much overrated if the counting of the exposure time starts already when the reference measurement point has overtaken the minimum sterilization temperature, even if at this moment not all the load has already entered the sterilization temperature band. In Annex 1, old Clause 92 and new Clause 8.52 are aimed to defend products against this specific risk.

Equilibration Time / Requirements II

EN ISO/TS 17665-1:2006, Clause 8.11:

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

a) *be* established by knowledge *of the bioburden (see Annexes B and C) or*

b) *be* determined by an 'overkill' method *(see Annex D) or*

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."

EN ISO 17665-1:2006, Definition 3.38, "Product family":

"groups or subgroups of product characterized by similar attributes *such as mass, material, construction, shapes, lumens, packaging system* and *which present* a similar challenge to the sterilization process".

Equilibration Time / Conclusions

Both “establishing” and “determining” and “demonstrating” an effective exposure to sterilizing conditions is based on biological challenge, even for a so-called “overkill” method. This refers to Cases a), b) and c) in the previous slide.

For Case d), Clause 8.11 of the Application Guidance CEN/ISOTS 17665-2 states an exception: *“If a product has been assigned to a product family for which a sterilization process has been defined and this sterilization process is based on an established time/temperature relationship, additional biological assessment is generally unnecessary”.*

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 that exceeds, in practice, the maximum value allowed for test loads shall be determined by biological challenge for any load and any loading pattern.

Moist-heat sterilization / 1 – Annex 1 2022

Clause 8.55: Moist heat sterilisation can be achieved using steam, (direct or indirect contact), but also includes other systems such as superheated water systems (cascade or immersion cycles) that could be used for containers that may be damaged by other cycle designs (e.g. Blow-Fill-Seal containers, plastic bags).

In this descriptive clause, an almost reticent wording refers to the so-called "counterpressure processes" for damageable containers.

These processes include the so-called "air-over-steam" ones; these processere are not mentioned at all in Annex 1 but are more and more frequently used for sterilizing not only the aqueous content of "difficult" containers, but also the external of them and the space between them and an outer envelope. The efficacy of a steam-air mixture as sterilant by contact can be demonstrated only by suitable and suitably located BIs.

Moist-heat sterilization / 2 – Annex 1 2008

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 …

Moist-heat sterilization / 2 – Annex 1 2022

For the new Clause 8.56 see above, "Avoiding recontamination" (slides no. 37 & 38).

Clause 8.57: For porous cycles (hard goods), time, temperature and pressure should be used to monitor the process and be recorded. Each sterilised item 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.

Clause 8.57 of Annex 1 2022 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".*

Moist-heat sterilization / 3 – Annex 1 2022

Clause 8.58: For autoclaves capable of performing prevacuum sterilisation cycles, the temperature should be recorded at the chamber drain throughout the sterilization period. Load probes may also be used where appropriate but the controlling system should remain related to the load validation. For steam in place systems, the temperature should be recorded at appropriate condensate drain locations throughout the sterilisation period.

Clause 8.58 of Annex 1 2002 converts a suggestion (“may be necessary”) *to the requirement of the recording of the temperature at the drain, if present,* “throughout the sterilization period” in the “autoclaves capable of performing prevacuum sterilisation cycles”. *This wording does not make clear* whether *this requirement applies also to cycles not including prevacuum phases but nevertheless performed in autoclaves capable of performing prevacuum.*

It is also stated very clearly that “load probes” cannot be used for controlling the process independently of the established load validation.

Moist-heat sterilization / 4 – Annex 1 2022

Clause 8.59: Validation of porous cycles should include a calculation of equilibration time, exposure time, correlation of pressure and temperature and the minimum/maximum temperature range during exposure. Validation of fluid cycles should include temperature, time and/or F_0. Critical processing parameters should be subject to defined limits (including appropriate tolerances) and be confirmed as part of the sterilisation validation and routine cycle acceptance criteria.

New Clause 8.59 of Annex 1 2022 defines the "essentials" of validation and specifies 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 / 5
Vacuum leak test / 2022 *vs* 2008

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

Clause 8.60: Leak tests on the steriliser 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 8.60 of Annex 1 2022 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.

It is hard to explain why the words "sterilizing system", that were used in Draft Version 12 and clearly included critical fittings as the air intake filter, have been replaced with a generic "steriliser".

Moist-heat sterilization / 6
Air removal test cycle / Annex 1 2022

Clause 8.61: There should be adequate assurance of air removal prior to and during sterilisation when the sterilisation 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 the use of 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.

Moist-heat sterilization / 6
Air removal test cycle / Comments

New Clause 8.61 of Annex 1 2022 describes another current "state-of-the-art" and formalizes that "the use of an air detector system" is considered equivalent to "an air removal test cycle (normally performed on a daily basis)". 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 / 7 – Annex 1 2022

Clause 8.62: Distortion and damage of non-rigid containers that are terminally sterilised, 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 (and established commercial "must") for sterilization of non-rigid containers.

Moist-heat sterilization / 8 – Annex 1 2022

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 sterilised. 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 sterilised by steam in place, it should remain integral and where operations require, maintained under positive pressure or otherwise equipped with a sterilising vent filter prior to use.

Clause 8.63 of Annex 1 2022 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 / 9 – Annex 1 2022 Superheated water autoclaves I

Clause 8.64: In fluids load cycles where superheated water is used as the heat transfer medium, 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.

Clause 8.64 of Annex 1 2022 describes the current "state-of-the-art" for superheated water sterilizers, thus demanding the effective distribution of the heating medium on the load, i.e. on all "the required contact points" of it.

Moist-heat sterilization / 9 – Annex 1 2022 Superheated water autoclaves II

Clause 8.65: Validation of the sterilisation of fluids loads in a superheated water autoclave should include temperature mapping of the entire load and heat penetration and reproducibility studies. All parts of the load should heat up uniformly and achieve the desired temperature for the specified time. Routine monitoring probes should be correlated to the worst case positions identified during the qualification process.

Clause 8.65 of Annex 1 2022 demands the actual attainment of the "desired temperature". For "fluids loads" time can be replaced by the equivalent time F_0 (see Clause 8.59 here above), thus the "specified time" can be the time required (and specified in validation studies) for achieving the desired F_0 target, but the effective attainment of a minimum declared temperature is unconditionally required. For the reliability of the temperature measured in routine by "in product" probes, see also Clause 8.52 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.

Clause 6.16: 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.

Clause 6.17: 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 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 (microbial testing is not mandatory for steam condensate). 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 (unless otherwise justified): non-condensable gases, dryness value (dryness fraction) and superheat.

Steam used as a direct sterilizing agent / Comment 1

Annex 1 2022 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 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 a current GMP in Pharma industry.*

Steam used as a direct sterilizing agent / Comment 2

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 / Sterility tests – 2022 *vs* 2008

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 critical 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 validation parameters. The test should be validated for the product concerned.

Clause 10.5 of Annex 1 2022 clearly explains that a product finally tested as sterile cannot be regarded as having been correctly sterilized. Accordance with the designed and qualified process is mandatory.

Quality control / Sampling – Annex 1 2008

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.

Quality control / Sampling – Annex 1 2022

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:

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. *[Relevant to products which have been filled lyophilized]*

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.

Quality control / Sampling – Comment

Clause 10.6 of Annex 1 2022 extends the examples to the case of lyophilization and strengthens the concept that samples shall be 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 /Annex 1 2022

Clause 10.10: 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.

Clause 10.10, as well as other clauses in Annex 1 20-22, is relevant to the organization of the Quality control. As such, they have an indirect yet meaningful impact on sterilization GMPs.

Parametric release / What it is - 1

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, 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".

Definition 2 (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".

Parametric release / What it is - 2

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 / 2022 *vs* 2008

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 8.54: 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 demanding enhanced bioburden assay and monitoring of the manufacturing process. Annex 1 2022 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 (for moist-heat sterilization)

As far as the moist-heat sterilization is concerned, Annex 1 2022 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 to the state-of-the-art.

The use of the Quality Risk Management approach is a general and challenging requirement of Annex 1 2022

An important alternative to the daily air removal test is offered by the routinely use of air detector system.

In spite of some ambiguities, the new text provides better descriptions of the requirements.

Thank you

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