Autoclaving in practice
The “ET issue”

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The sterilization autoclave

Both thermal and chemical and filtration actions are suitable for sterilization. Thermal sterilization may be obtained by dry heat or moist heat

A Sterilization Autoclave is a Pressure Vessel intended to perform a thermal **S**terilization Process, i.e., the complete inactivation of the viable micro-organisms initially present inside pharmaceutical products *for human or animal use,* and / or on the external and internal surfaces of items to be used in health care or pharmaceutical production environments

The thermal process performed is autoclaves is the *Moist-heat sterilization*

Physical conditions for moist-heat sterilization

According to *USP 43, <1229.1>*, Moist-heat sterilization *"rapidly kills microorganisms because of the presence of liquid water."* The process temperature is generally between 110 °C and 140 °C, but sterilization temperatures higher than 134 °C are unusual

The liquid water may be originally present in the product or obtained from condensing steam. In the second case, the process may be called "steam sterilization by direct contact"

Depending on the type of load, this involves a process pressure between 1.5 and 5.5 abs bar, i.e., between 0.5 and 4.5 bar gauge at sea level

Autoclaves rated for more than 3 bar gauge are unusual

Types of load

PDA TR No.1 Revised 2007, Section 2.0, "Glossary of Terms":

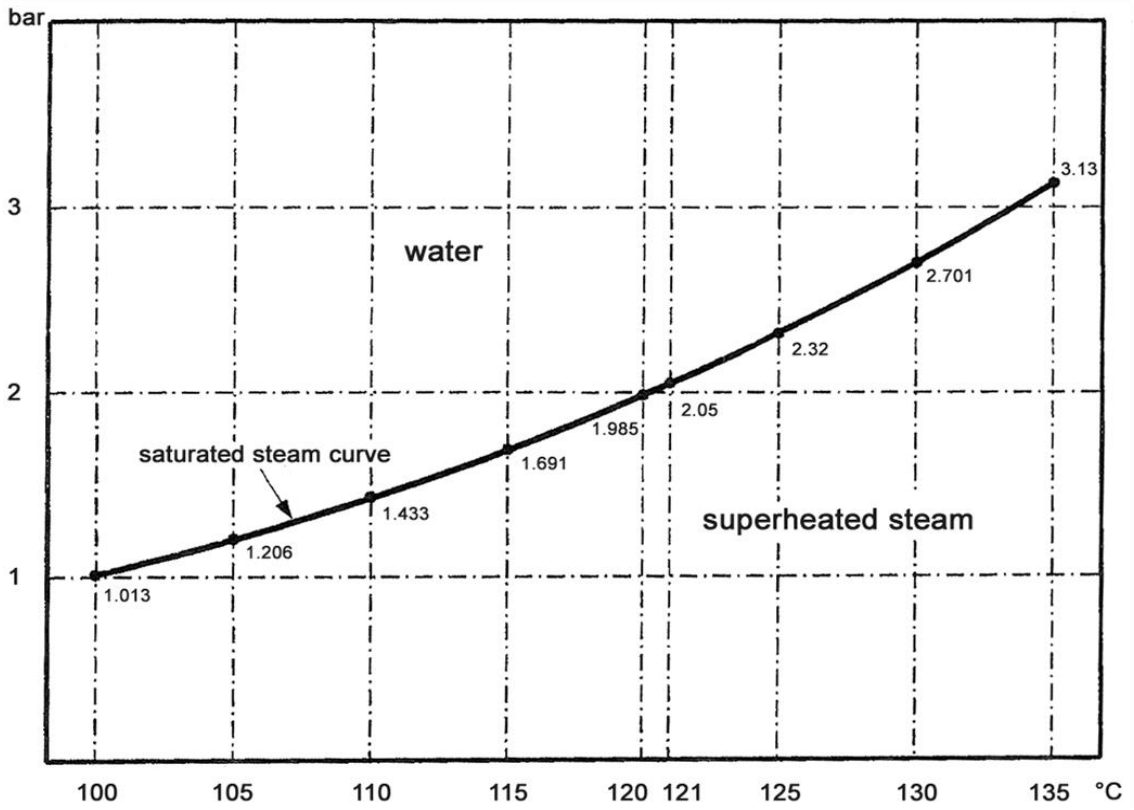
Porous/Hard Goods Load (P/HG): A porous/hard goods load consists of items in which the bioburden is inactivated through direct contact with saturated steam. Porous/hard goods load items include: filters, stoppers, tubing (hoses), mops, garments, stoppers, cleaning equipment, or machine change parts.

Liquid Load: A load consisting of closed containers of aqueous liquids. The sterilization of the container contents is achieved through transfer of energy through the container into the aqueous liquid.

HTM 01-01 Part C, paragraph 1.35, a:

*… **porous loads trap both air and moisture***

Water state chart



PSS or contact sterilization (I)

In pure saturated steam sterilization, the necessary intimate *contact* between condensing steam and the microorganisms to inactivate on the surfaces of a “porous/hard goods load” demands for the total removal of the air initially surrounding the load

Thus, prior to the heating and sterilization phases, it is necessary to remove completely the air from the chamber and from the surface to sterilize

This removal is generally (and in some cases mandatorily) obtained by a vacuum system (usually a vacuum pump) connected with the autoclave

The steam fed to the autoclave shall be Pure Saturated Steam of *known, suitable and repeatable quality.* In PSS sterilization, the steam fed into the chamber shall both heat the load and sterilize its exposed surfaces at the specified conditions

PSS or contact sterilization (II)

During the exposure phase of *pure saturated steam (PSS) sterilization processes*, no appreciable quantity of any gas or vapor is expected to be longer present in the sterilizer

In these conditions, thanks to the so-called *Phases' rule*, the level of the process pressure is dictated one-to-one by the choice of the sterilization temperature

Finally, drying and / or cooling phases are performed, to allow a safe extraction of the sterilized items from the autoclave and their preservation under sterile conditions.

PSS processes are mainly used to sterilization of porous/hard goods, but they may also be for tightly sealed small vials and ampoules filled with solutions to be terminally sterilized. They *are not suitable for ampoules with movable plungers and for large volume containers filled with liquids*

PSS sterilizers

HTM 01-01 Part C, paragraphs 1.34 to 1.36:

"Sterilizers using high temperature steam to process porous loads are commonly known as "porous-load sterilizers". Porous loads are typically "*towels, gowns and dressings, plus medical and surgical equipment, instruments and utensils that are packaged or wrapped in porous materials such as paper, fabrics or sterilization containers with filters."*

"Porous-load sterilizers are distinguished from other high-temperature steam sterilizers by the following features:

a. … a vacuum system to ensure sufficient air removal prior to steam supply, and final drying

b. an air detector … to ensure that the plateau period cannot start until sufficient air has been removed from the chamber

c. a heated jacket … to prevent condensate from forming on the chamber walls and to assist drying of the load."

"Porous-load sterilizers should conform to the specifications in BS EN 285 and the safety specifications in BS EN 61010-2-040."

Equilibration time (ET)

Why air must be removed from the chamber?

Dry heat relies solely on thermal destruction of the membrane and slowly attacks the cells layer by layer

Moist heat also hydrates the outer membrane of cells, helping heat penetration and destroying them quicker

To ensure moist-heat conditions throughout the load
the air must be removed in a uniform way and the load must be heated by condensing steam

Equilibration Time is a measure of all this

Reference standards and guidelines for ET

HTM 2010 - Part 3: Sterilization – Validation and verification
(superseded by *CFPP 01-01, now HTM 01-01 - Part C*)

HTM 01-01 - Part C: Steam Sterilization

EN 285:2015: Sterilization - Steam Sterilizers - Large Sterilizers

PDA TR No.1, Revised 2007: Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control

EN ISO 17665-1:2006: Sterilization of health care products – Moist heat – Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices

Superseded definitions of ET

HTM 2010 Part 3, Glossary:

"The period which elapses between the attainment of the sterilization temperature in the chamber and the attainment of the sterilization temperature in all parts of the load."

An explanation was given in HTM 2010 Part 3, Clause 7.10:

"A period in which the sterilization conditions are present in the chamber but not yet present throughout the load."

EN 285:1996 (1st edition):

"Period which elapses between the attainment of the sterilization temperature in the sterilizer chamber and the attainment of the sterilization temperature at all points within the load."

Both these definitions were unsatisfactory because *there are always some temperature differences inside a sterilizer chamber*

Current definitions of ET

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”

According to EN 285:2025, Clause 25.3 f) 6), *“the location of the reference measurement point” “shall be available* [among a lot of other information] *upon the sterilizer delivery”*

Shared concepts on ET (I)

HTM 01-01 Part C, Clauses 2.85 & 2.86:

"The equilibration time begins when the temperature in the reference point (that is, the point where the cycle control temperature sensor is situated) first attains the sterilization temperature. It ends when the holding time begins."

"The holding time begins when the temperature in the part of the load that is the slowest to heat up first attains the sterilization temperature. It ends at the start of the cooling stage, when the temperature in the coolest part of the chamber falls below the sterilization temperature."

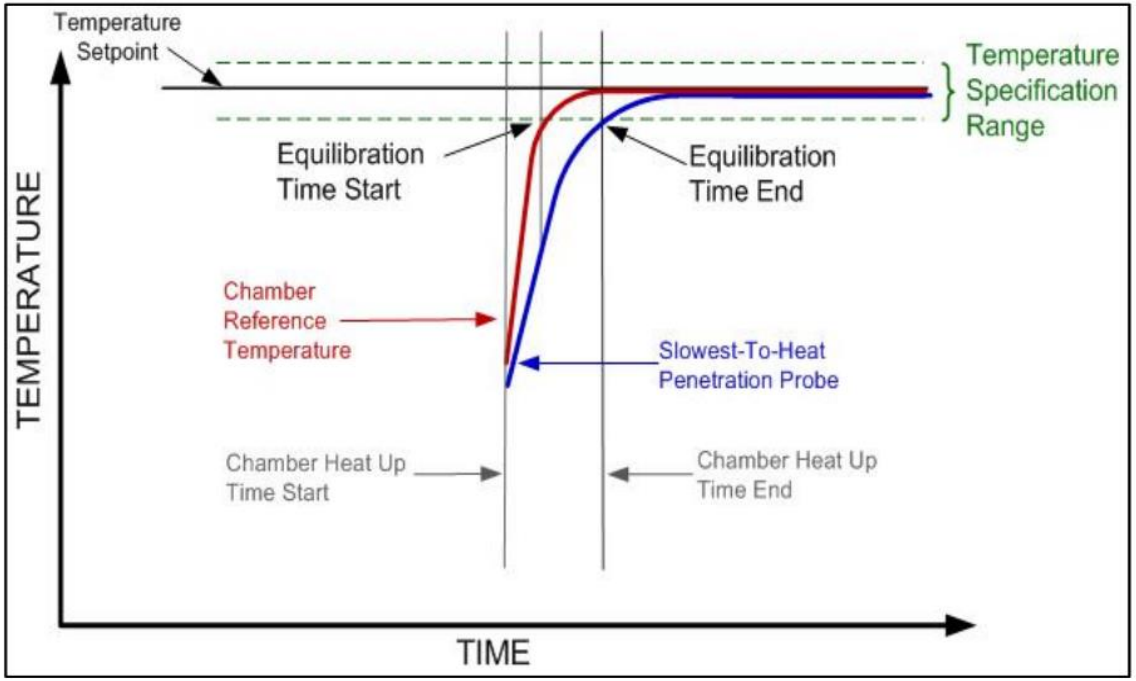
PDA TR No. 1 Rev. 2007, Section 2.0, "Glossary of Terms":

"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 by placing heat penetration probes** in porous/hard goods loads."

Shared concepts on ET (II)

PDA TR No. 1, Revised 2007, Section 4.4.1.5, "Equilibration Time":

"*Equilibration time is an important function of conditioning porous/hard goods loads that includes the number and depth of prevacuum and positive pulses. The figure graphically depicts equilibration and chamber heat-up time*."



ET: requirements for sterilizers

EN 285:2015, Clauses 8.2.1.2.1 (Small load) & 8.2.1.3.1 (Full load):

"The equilibration time shall not exceed 15 s for sterilizer chambers up to 800 l usable space and 30 s for larger sterilizer chambers ."

EN 285:2015, Clause 8.2.1.3.2 (specific to Full load):

"At the end of the equilibration time, the temperature measured at the reference measurement point of the sterilizer chamber and the temperature measured at the nominal geometric centre and *below the top sheet* of a standard test pack (see 23.1) located in the test load shall be within the sterilization temperature band."

Scope of requirements for ET

The scopes of an upper limit for the equilibration time are apparent:

to prevent that the desired temperature is eventually achieved by heat transmission instead of steam penetration; this would make uncertain the presence of liquid water on the surfaces to sterilize

to avoid an excessive overrating of the effective exposure time (“holding time”); in fact, the calculation of the exposure time usually begins when the reference measurement point has overtaken the minimum sterilization temperature, even if at this moment not all the load has already done the same

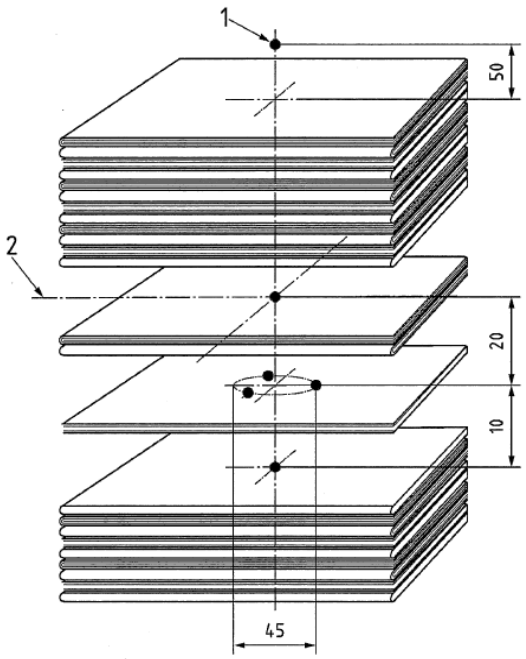
*The requirements for Equilibration Time not exceeding 30 (or 15) seconds are referred to **test loads**. The requirement on Equilibration Time duration is part of specification of the sterilizer and has the aim to demonstrate, by mean of the standard test load, that a sterilizer or a type of sterilizers is compliant with the Standard*

Test loads for ET requirements

Standard Test Pack (STP), *and how to prepare and use it, is described in detail in EN 238:2015, Clauses 23.1.*

Clauses 23.2 are devoted to Reduced Test Pack*, intended for "one module" sterilizers (a sterilization module is a " rectangular parallelepiped of dimensions 300 mm (height) x 600 mm (length) x 300 mm (width)"*

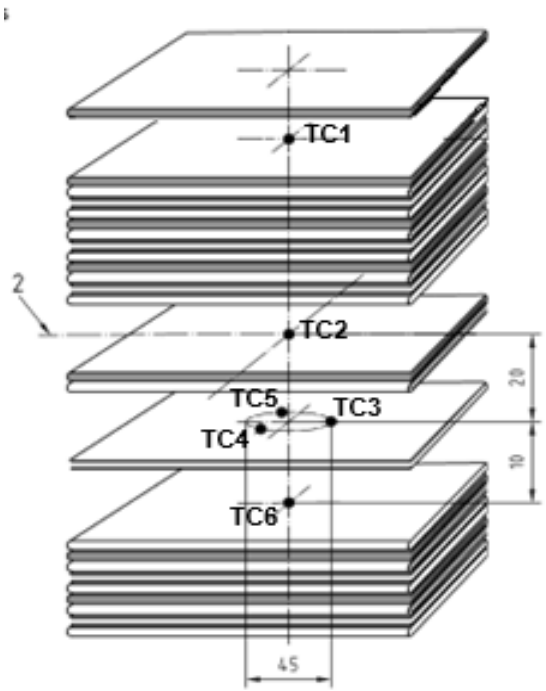
TC-probes positions for ET tests (I): Small load



EN 285:2015, Chapter 16 deals with thermometric tests of operating cycles of sterilizers. Seven test probes independent of the control system are required: one always at the reference measurement point (Clauses 16.1.3.3 & 16.1.2.3.3) and six located in and above the STP

This figure refers to the "Small load": the positions of TCs are defined by Clauses 16.1.3.6 & 16.1.3.8

TC-probes positions for ET tests (II): Full load



EN 285:2015, Chapter 16 deals with thermometric tests of operating cycles of sterilizers. Seven test probes independent of the control system are required: one always at the reference measurement point (Clauses 16.1.3.3 & 16.1.2.3.3) and six located in the STP

This figure refers to the "Full load": the positions of TCs are defined by Clause 16.2.3.6

In comparison with Small load, TC1 is no longer 50 mm above the STP, but just under the top sheet

ET: requirements for actual sterilization processes (I)

EN ISO 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"

ET: requirements for actual sterilization processes (II)

No other reference to ET may be found in any part of EN ISO 17665:2006, but Part 2 - 8.11 specifies that overkill method is a *"sterilization process bases on a defined biological challenge based on biological indicators"*

By comparing this statement with the list of Clause 8.11 of Part 1, it is apparent that only biological indicators provide an escape way form the respect of restrictive limits for Equilibration Time. In fact, BIs are essential both to cases a) and b) (knowledge of the bioburden or overkill method), and, as in case c), for *"demonstrating that during the holding time all parts of the product are exposed to process parameters*", which in turn must follow official pharmacopoeias

On the other side, it is requested to demonstrate that the actual loads are comparable with loads (either test loads or a validated product family) for which a maximum ET has been assigned

ET: a summary of concepts

PDA TR No. 1 Revised 2007, Section 4.4.1.5 (cont.):

"…The equilibration time is the period that elapses between attainment of the minimum specified sterilizing temperature in the chamber (chamber reference point – typically the drain) and attainment of the minimum specified sterilization temperature in the load, as measured by the slowest-to-heat penetration probe. This period is an indication of the ability to properly condition the load through air removal and load heating.

***Extended equilibration times can be indicative of inadequate air removal or heating, even if the desired temperature is eventually achieved.**"*

ET: an example

Stopper bags

TC02

ET: poor air removal and poor drying

```
1 PREPARE AUTOCLAVE                          1
2 DEPRESSURIZE BY VACUUM PUMP                9
3 HEATING                                   41
4 STERILIZATION                             41
5 DEPRESSURIZE BY VACUUM PUMP                9
6 TIMED VACUUM                              21
7 RETURN TO ATMOSPHERIC PRESSURE           137
8 CYCLE END                                148
9 EMERGENCY                                157
```

Temperature Data(°C)	TC 01 °C SIM 1	TC 02 °C SIM 1	TC 03 °C SIM 1	TC 04 °C SIM 1
20-Feb-2019				
11:25:50	110.20	76.80	117.20	120.40
11:25:55	110.50	78.40	118.70	120.40
11:26:00	110.90	79.80	119.50	120.40
11:26:05	111.30	80.80	119.80	120.40
11:26:10	111.80	81.90	120.10	120.40
11:26:15	112.60	82.80	120.20	120.40
11:26:20	113.60	83.70	120.30	120.40
11:26:25	114.80	84.80	120.40	120.50
11:26:30	116.10	86.00	120.40	120.50
11:26:35	117.20	87.20	120.40	120.60
11:26:40	118.20	88.10	120.50	120.60
11:26:45	119.00	89.10	120.50	120.60
11:26:50	119.50	90.40	120.50	120.60
11:26:55	119.90	91.40	120.50	120.60
11:27:00	120.10	92.70	120.60	120.60
11:27:03	*** Start Exposure *** *******Exp Start*******			
11:27:03	120.30	93.40	120.60	120.60

ET: effective air removal and drying

PHASE LISTS

n.	phase	group n.
1	PREPARE AUTOCLAVE	1
2	AIR TO CHAMBER	123
3	REDUCED RATE DEPRESSURIZE	10
4	NORMAL RATE DEPRESSURIZE	10
5	MODULATED STEAM RISING PULSE	32
6	DYNAMIC STEAM PRESSURE HOLD	32
7	FALLING PULSE SLOW	32
8	FALLING PULSE NORMAL	32
9	STEAM TO CHAMBER, MODULATED	45
10	STEAM TO CHAMBER, MODULATED	42
11	STERILIZATION	42
12	REDUCED RATE DEPRESSURIZE	10
13	NORMAL RATE DEPRESSURIZE	10
14	TIMED VACUUM	21
15	RISING AIR PULSE	121
16	AIR PRESSURE HOLD	121
17	FALLING PULSE	121
18	TIMED VACUUM	121
19	MODULATED VACUUM BALANCE	138
20	CYCLE END	148
21	EMERGENCY	156

Temperature Data(°C)

	TC 01 °C SIM 1	TC 02 °C SIM 1	TC 03 °C SIM 1	TC 04 °C SIM 1
14-Feb-2019				
11:21:05	115.10	115.30	115.20	115.20
11:21:10	117.20	117.30	117.20	117.20
11:21:15	118.80	118.90	118.90	118.80
11:21:20	119.50	119.60	119.50	119.40
11:21:25	120.10	120.10	120.10	120.00
11:21:30	120.50	120.50	120.50	120.40
11:21:35	120.80	120.80	120.80	120.70
11:21:40	121.00	121.10	121.10	121.00
11:21:45	121.30	121.30	121.30	121.20
11:21:50	121.40	121.50	121.50	121.40
11:21:55	121.60	121.60	121.60	121.50
11:22:00	121.70	121.70	121.70	121.70

ET as cycle development criterion (I): PDA

PDA TR No. 1, Revised 2007, Section 5.3,
"Process Performance Acceptance Criteria":

"Acceptance criteria … should be based [among other] *on the operating parameters determined in cycle development."*

"Maximum equilibration time" is listed among the *"typical physical qualification acceptance criteria".*

ET as cycle development criterion (II): UK

HTM 01-01 Part C, paragraph 1.36,
"Porous load sterilizers, Standard specifications":

"Porous-load sterilizers should conform to the specifications in BS EN 285"

HTM 01-01 Part C, paragraphs 4.13 and 4.14,
"Cycle variables":

"Settings for the automatic controller will be determined during performance qualification.
Generally, these will consist of a chamber temperature within the sterilization temperature band and a ***plateau period designed to accommodate the equilibration time and the holding time****."*

Formally, no upper limit can be found in HTM 01-01 for equilibration time of sterilization *processes*, but only for testing a sterilizer for compliance with EN 285

ET: suggestions

PDA TR No. 1 Revised 2007, Section 4.4.1.5 (cont.):

"When developing a cycle, it is important to take practical precautions to minimize equilibration time.

The following options can be used to reduce the Equilibration time:

- Assure loads are oriented for efficient air removal (e.g. hoses not pinched)
- Increase number of vacuum or positive pulses
- Add hold steps during vacuum and/or steam pulses
- Increase depth of vacuum pulses
- Optimize steam exposure to load items"

The current practice of sterilization demonstrates that even for very difficult loads the equilibration time can be reduced by suitable air removal steps to the low values foreseen by EN 285:2015 for test loads.

Thank you

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