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Load Types, Sterilization Processes & Autoclaves

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PDA® Parenteral Drug Association

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Agenda

Introduction

Load types, Sterilization processes & Autoclaves

Saturated Steam Autoclave

Generality & cycle description

Counterpressure Autoclaves

Generality & cycle description

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Load types, Sterilization processes & Autoclaves

Saturated Steam Autoclave

Generality & cycle description

Counterpressure Autoclaves

Generality & cycle description

What is an autoclave?

A Sterilization Autoclave is a **Pressure Vessel intended to perform a Sterilization Process**, i.e., the *complete inactivation of all viable micro-organisms* 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.

Load types: **POROUS/HARD GOODS**

Items where sterilization is achieved through **direct contact with saturated steam**.

Heat is transferred when steam condenses directly on the surface of the items being sterilized.

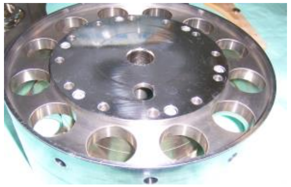
Porous/hard goods loads, as used in the pharmaceutical industry, encompass true ***porous items*** (such as cartridge filters and packed fabric) and ***hard items*** (such as stainless-steel vessels and filling machine parts).

PDA TR No. 1- 4.2 Load Types









Load types: **LIQUID**

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.

Examples of liquid-filled containers include, but are not restricted to: ***formulations*** (solutions, suspensions and/ or emulsions) in their final product container (e.g., vial, bag, bottle, syringe or ampoules), ***post-test*** or ***post-process waste fluids*** containing potentially pathogenic microorganisms.

PDA TR No. 1- 4.2 Load Types

Sterilization processes & Autoclave types

- <u>**Saturated steam sterilization**</u>

  A sterilization process, typically used for *porous/hard goods loads*, where the sterilizing medium is *saturated steam*.

Saturated Steam Autoclave [FOF]

- <u>**Counterpressure sterilization**</u>

  Steam-Air Mixture Process: A sterilization process in which the heating medium used to heat the load is in a *mixture of air and steam* that is typically used for *liquid loads*. This addition of air results in an *air overpressure condition*.

Steam Air Mixture Sterilizer [FOA]

  Superheated Water Process*:* A sterilization process in which the heating medium is *superheated water* that is continuously circulated with *air overpressure*.

Water Cascade Sterilizer [FOW]

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Saturated Steam Autoclave

Generality & cycle description

Counterpressure Autoclaves

Generality & cycle description

Sterilization by direct contact

A sterilization process, typically used for **porous/hard goods** where the sterilizing medium is **saturated steam**.

PDA TR N. 1, Glossary

Saturated steam is *"water vapour in a state of equilibrium between condensation and evaporation"*

UNI EN ISO 17665-1

The initial objective for saturated steam sterilization is that the **air** in the sterilizing chamber **must be replaced by saturated steam**.

USP 43 Chapter 1229.1

- Residual air acts as an **insulator**
- The presence of residual air in the chamber **negates the singular temperature–pressure relationship of saturated steam**

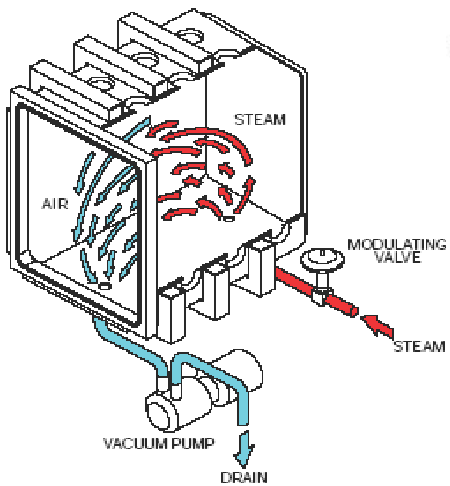
Fedegari Saturated Steam Autoclave (FOF)

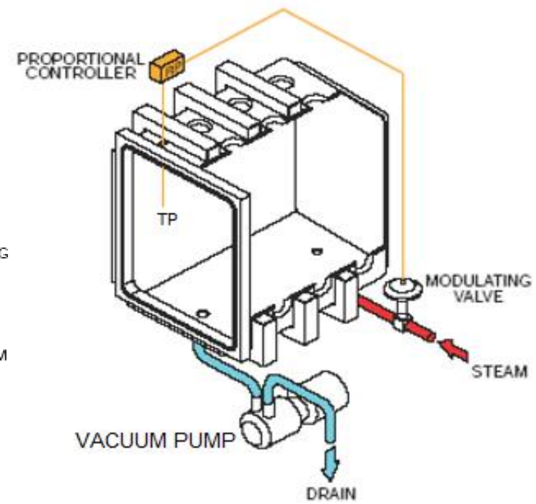
Fedegari Horizontal Pharmaceutical is the most flexible solution for **multi-purpose sterilization** in bio-pharma industries.

From **solids** and **porous** to **liquids** in open or non-hermetically sealed containers.

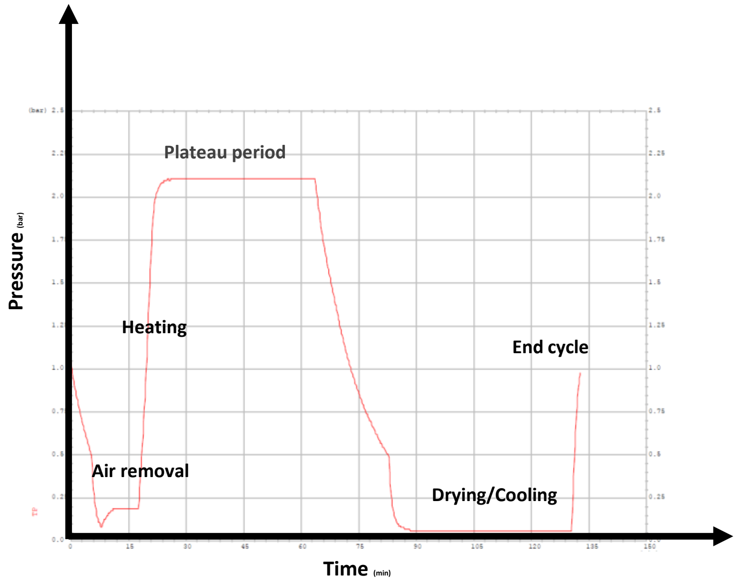
Fedegari Saturated Steam Autoclave (FOF)

The **sterilization temperature is controlled according to a pressure signal**, thanks to the one - to - one correspondence of temperature and pressure for the pure saturated steam.



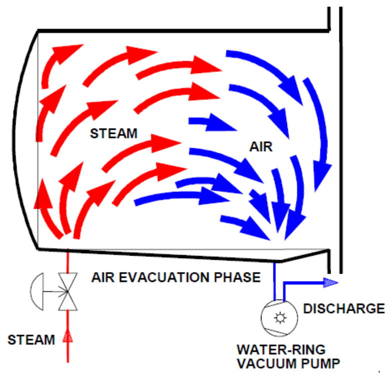


Process phases



1. **Preheating** with hot air (usually for loads very hard to dry) or other auxiliary treatments
2. **Air removal** from the chamber (e.g. by vacuum)
3. **Heating** & **Sterilization**
4. **Post-sterilization phases** (drying and/or cooling)

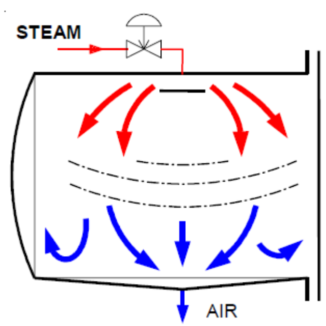
Initial air removal from the chamber



- ***Depressurization plus steam injection***
  (e.g. metal items, empty glassware open)

- ***Steam/ vacuum pulses***
  (e.g. porous solids where air removal is critical: filters, textiles, stoppers in bag, hollow materials)

→ These methods removes air from the chamber using a mechanical **vacuum pump** or steam eductor.

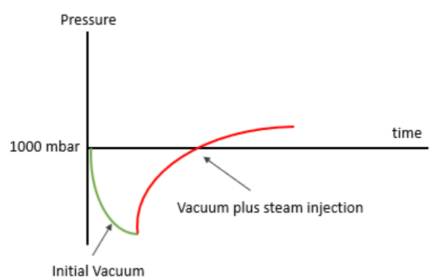


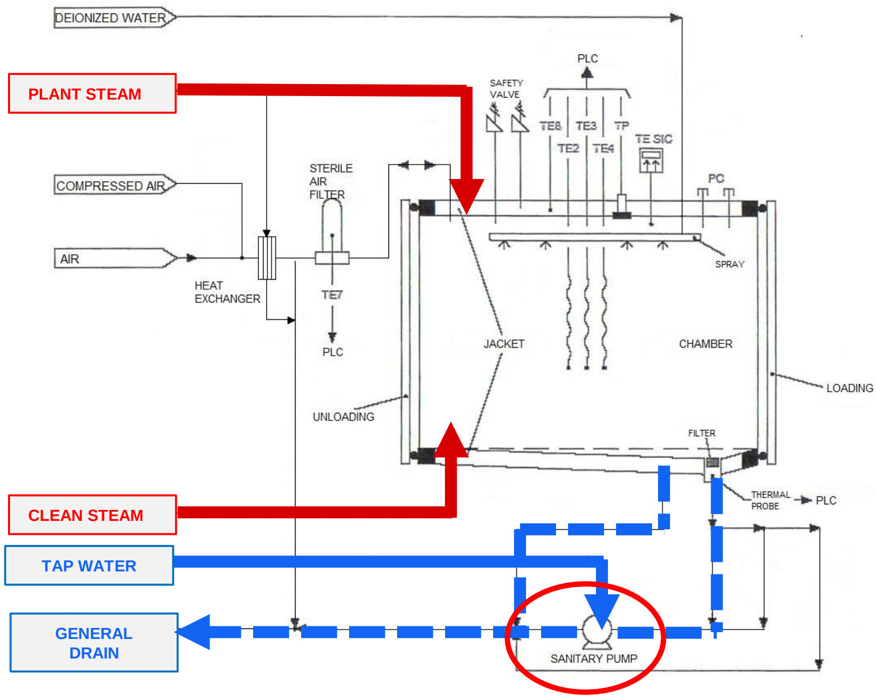
- ***Displacement by Gravity (old autoclaves)***

Depressurization plus steam injection

Once the initial vacuum has been reached, steam is injected for a few minutes while the vacuum pump is kept continuously running (P constant).

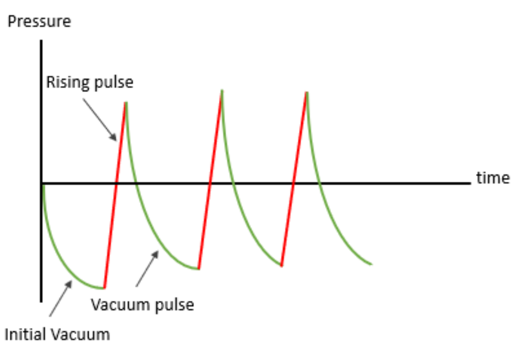
Vacuum plus steam injection

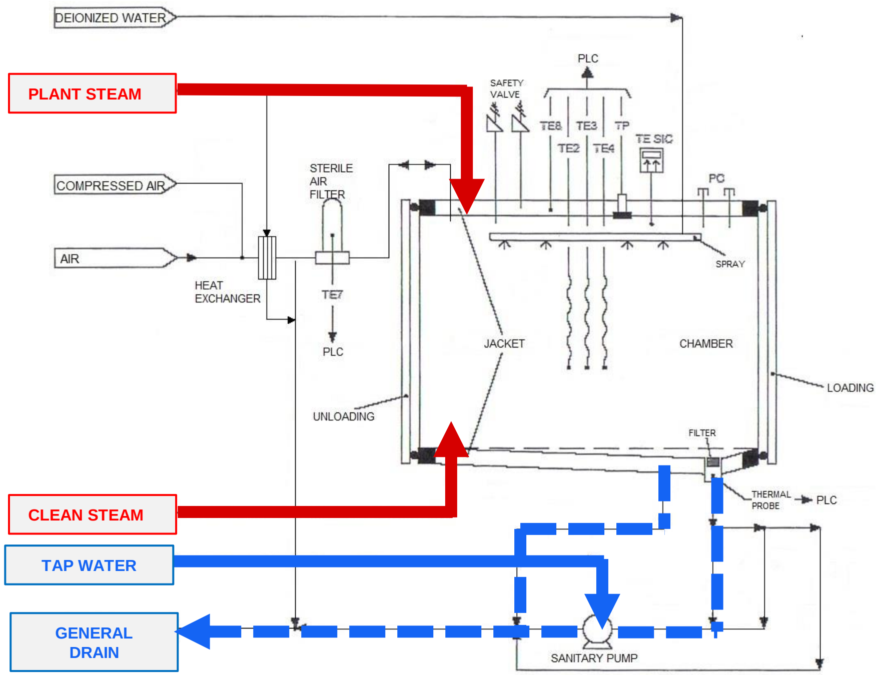




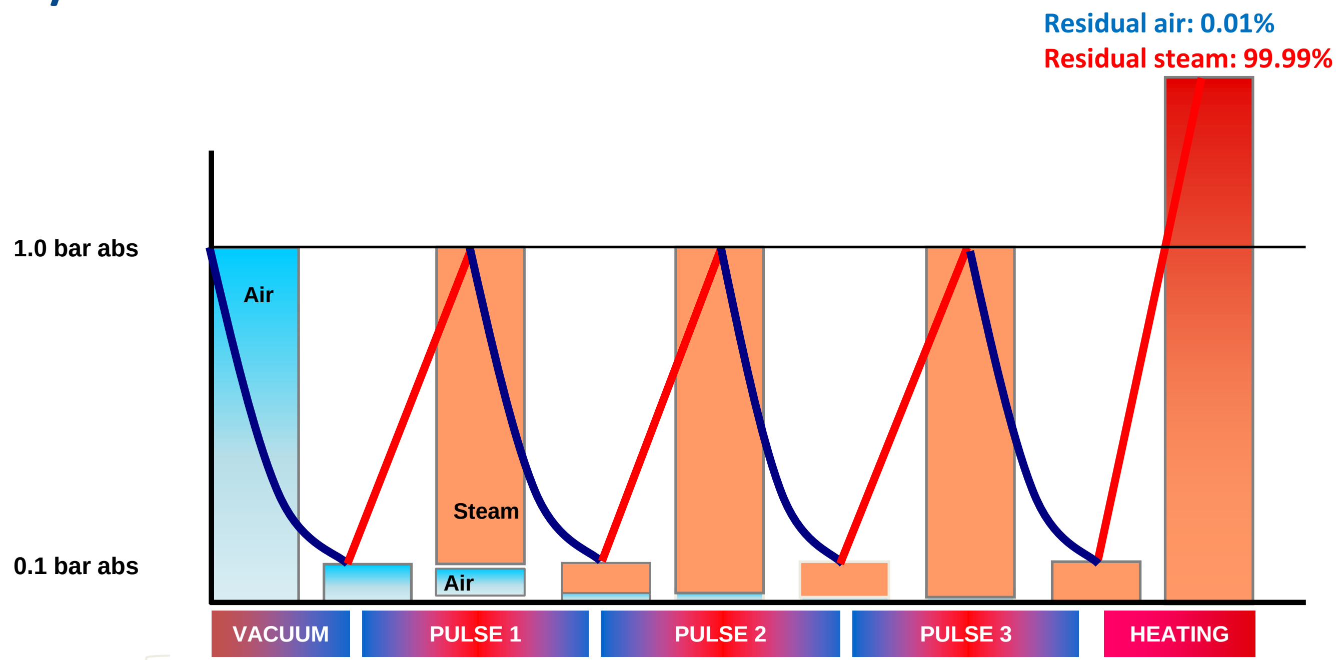
The **vacuum pump** extracts both steam/condensate and air from the chamber

Steam/ vacuum pulses



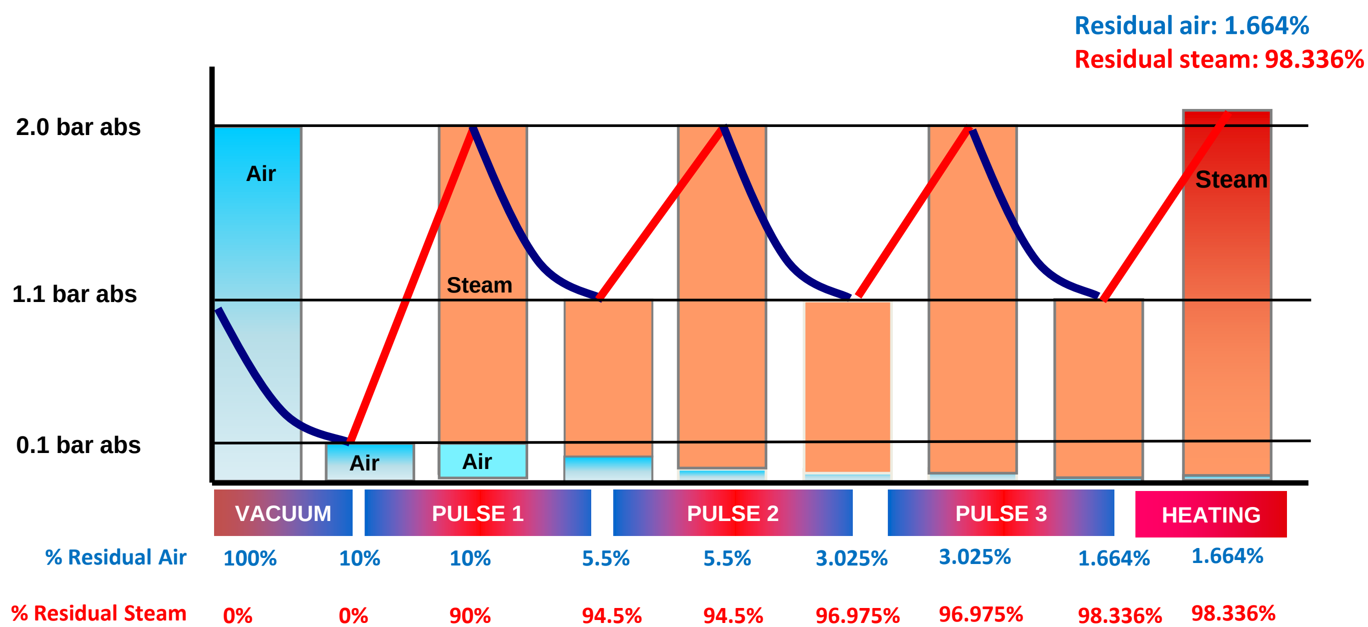


Negative Steam/Vacuum Pulses

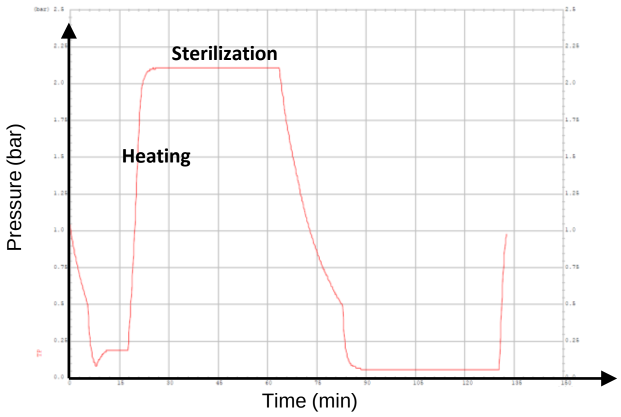


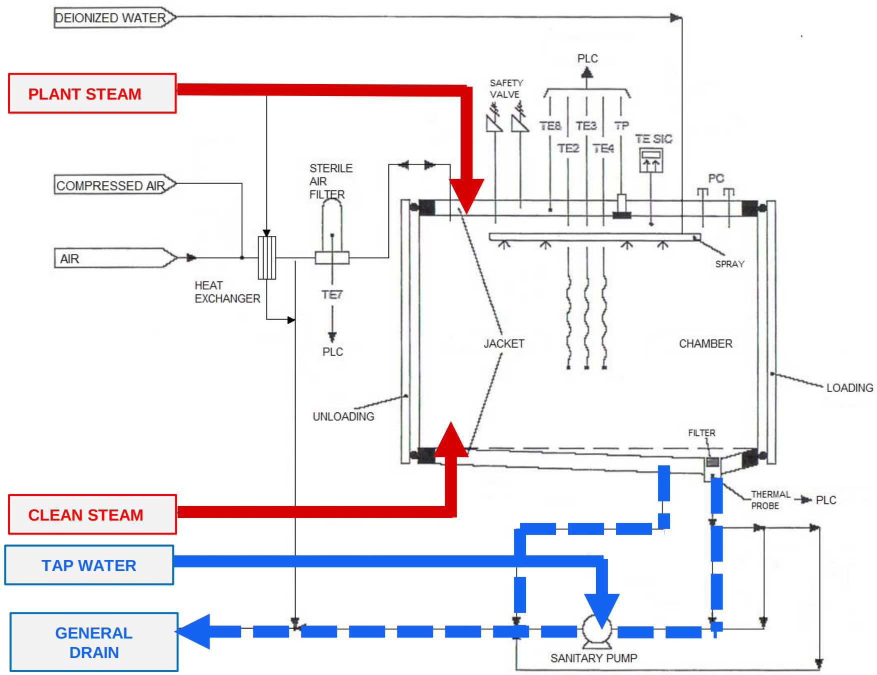
Theoretical partial pressure: Air	1.0	0.1	0.1	0.01	0.01	0.001	0.001	0.0001	0.0001
Theoretical partial pressure: Steam	0.0	0.0	0.9	0.09	0.99	0.099	0.999	0.0999	0.9999
% Residual Air	100%	10%	10%	1%	1%	0.1%	0.1%	0.01%	0.01%

Positive Steam/Vacuum Pulses



Heating & Sterilization





Post sterilization phases

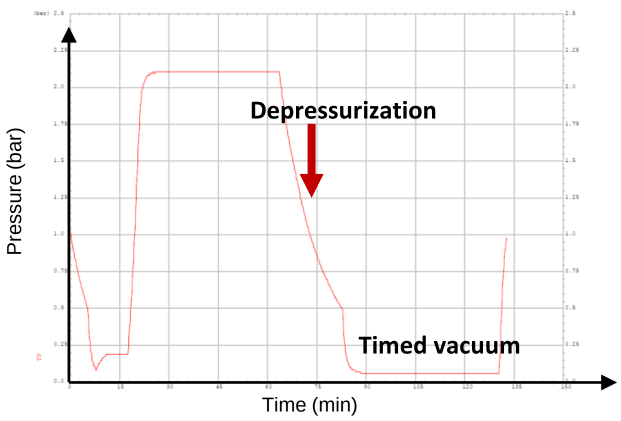
Drying:

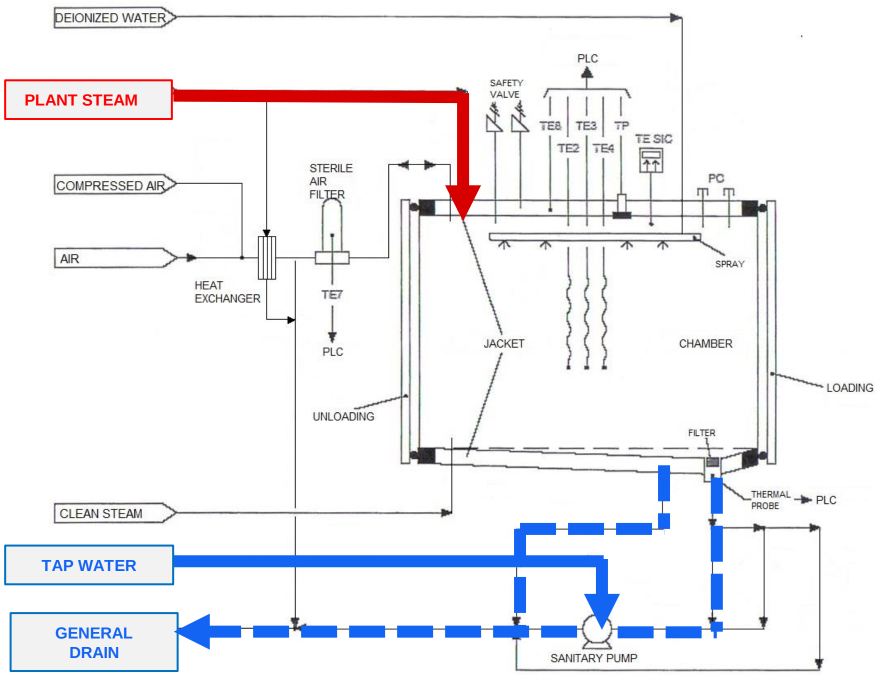
- vacuum and time-controlled/ vacuum hold
- air pulses

Cooling:

- indirect cooling
- direct cooling

Vacuum and Time-controlled/ Vacuum hold



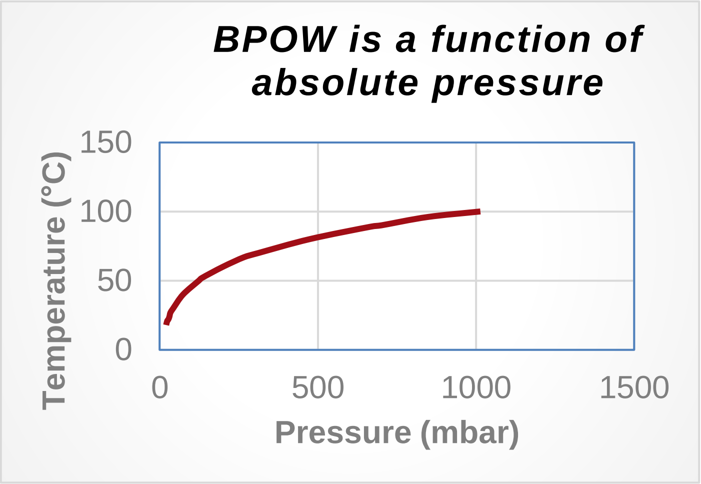


Vacuum and Time-controlled/ Vacuum hold

Drying and "natural" cooling by final vacuum

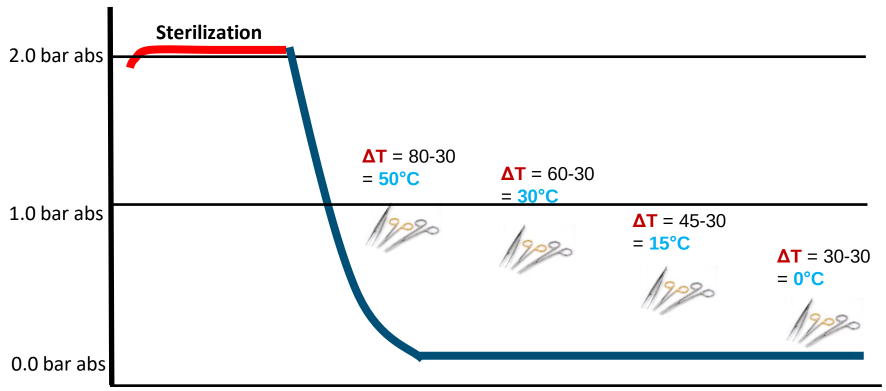
By creating a vacuum in the chamber, we are able to **lower the boiling point of water (BPOW)**.

We are able to create a **temperature differential between the BPOW and the load item**, which is hot due to the sterilization phase already performed



mbara	degC
1013.3	100
846.6	96
700.6	90
666.6	89
473.4	80
311.5	70
266.6	67
199.1	60
133.3	52
123.3	50
73.48	40
42.33	30
33.86	27
30.48	24
27.09	22
23.71	21
20.32	18

Vacuum and Time-controlled/ Vacuum hold

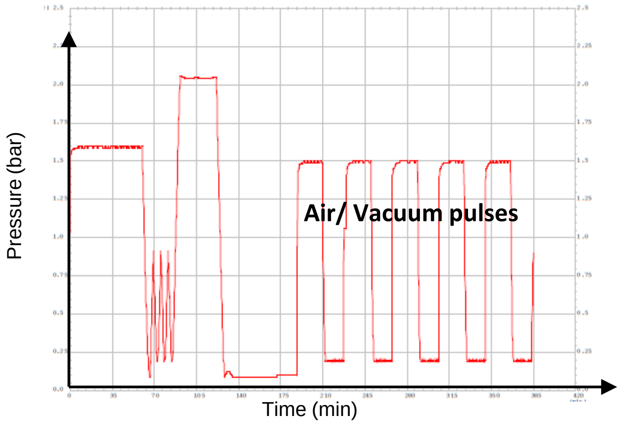


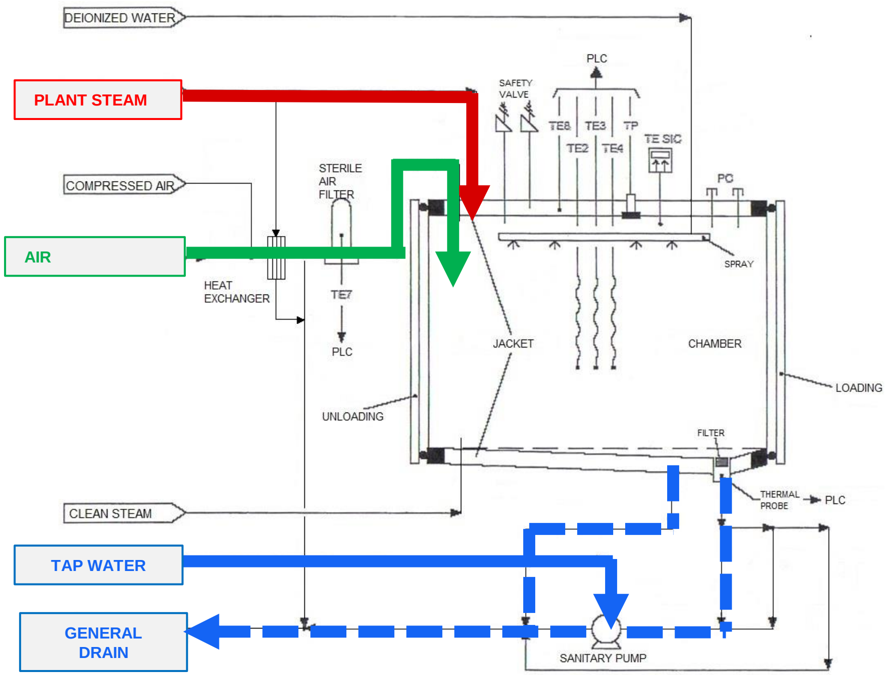
During ‘*vacuum drying*’, we are limited in our ability to add extra ‘heat’ into the load to reduce the drop in load temperature.

As the load cools, there will come a point when no more evaporation is possible, as the load temperature matches the boiling point of water

At this point, if the load hasn’t reached the desired ‘dryness’ additional processing is required.

Hot air – Vacuum pulses





Cooling

- **Indirect cooling**
  - ✓ water circulation in the jacket
  - ✓ air counterpressure
- **Direct cooling**
  - ✓ spray of sterile water onto the load

Case studies

- Stopper in bags
- Small Volume Parenteral in sealed containers
- Mixed Load

Stoppers in bags

Load description

Rubber stoppers in tyvek/ plastic bag

Customer request

Residual humidity $\leq$ 0,1 %

Autoclave type

Saturated steam sterilizer (FOF)

Stoppers in bags

Solid load drying: key points

- Material design & packaging system
- Item orientation/arrangement
- Drying by vacuum improved by:
  - Auxiliary heating equipment
  - Vacuum / (hot) air pulses
  - Forced circulation of hot air (with fan)

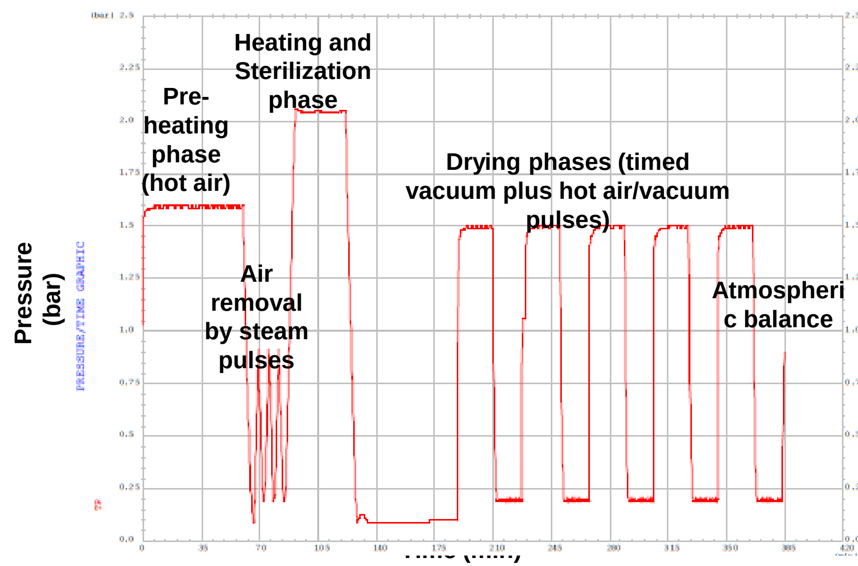
Stoppers in bags

Load pre-heating to reduce condensate production.

Air removal by modulated depressurization followed by modulated steam/ vacuum pulses

Drying by vacuum/air pulses

PHASE LISTS
n. phase
1 PREPARE AUTOCLAVE
2 AIR TO CHAMBER
3 MODULATED DEPRESSURIZATION
4 TIMED VACUUM
5 MODULATED STEAM RISING PULSE
6 DYNAMIC STEAM PRESSURE HOLD
7 MODULATED FALLING PULSE
8 TIMED VACUUM
9 STEAM TO CHAMBER, MODULATED
10 STERILIZATION
11 MODULATED DEPRESSURIZATION
12 TIMED VACUUM
13 RISING AIR PULSE
14 AIR PRESSURE HOLD
15 FALLING PULSE
16 TIMED VACUUM
17 RETURN TO ATMOSPHERIC PRESSURE
18 CYCLE END
19 EMERGENCY



Stoppers in bags

Relative Humidity evaluation

Gravimetric method to determine RH
Stoppers are weighed before and after a specified drying procedure at a defined temperature.

$$\text{Residual Humidity (\%)} = \frac{(\text{Wet weight} - \text{dried weight}) \times 100}{\text{Wet weight}}$$

Result

RH ≅ 0,03%

Small Volume Parenteral in sealed containers

A **Large volume parenteral** (LVP) is a unit dose container of greater than 100 ml that is terminally sterilized by heat.

A **Small volume parenteral** (SVP) is a unit dose that is packaged in containers labeled as containing 100 mL or less.

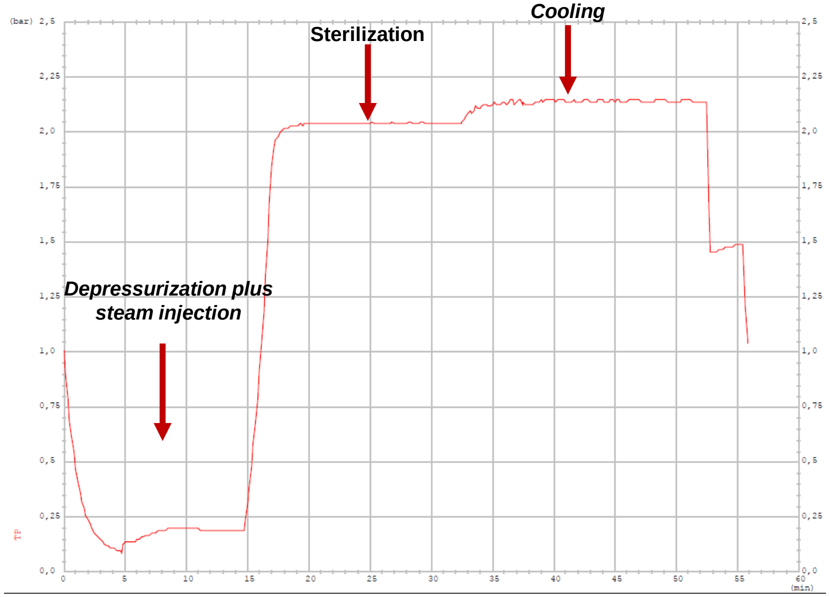
Small Volume Parenteral in sealed containers

Air removal method: vacuum plus steam injection

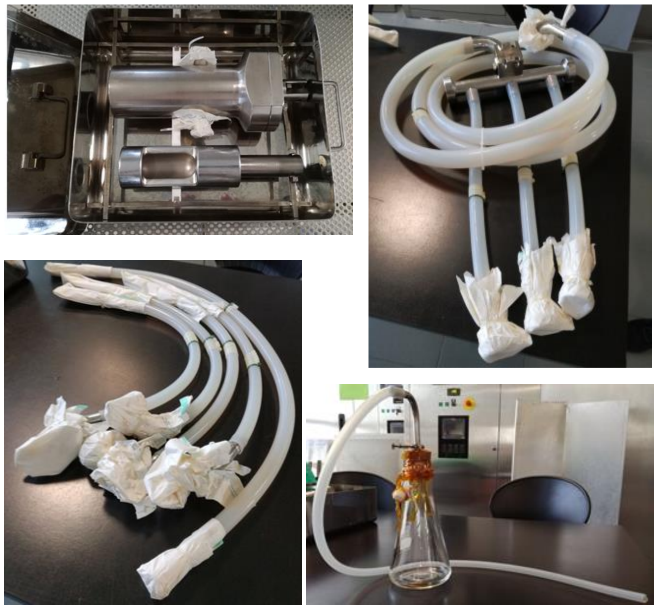
Heating & Sterilization

Cooling method: indirect cooling

1	PREPARE AUTOCLAVE	1
2	DEPRESSURIZE BY VACUUM PUMP	9
3	TIMED VACUUM, STEAM INJECTION	20
4	HEATING	41
5	STERILIZATION	41
6	PRESSURIZE CHAMBER BY AIR	81
7	CONTROLLED RATE COOLING	81
8	COOLING EXTENSION	81
9	WATER DRAIN	81
10	RETURN TO ATMOSPHERIC PRESSURE	137
11	CYCLE END	148
12	EMERGENCY	157



Mixed Load



Load description

- A metal box containing a dosing pump with relative piston
- A metal basket containing 4 needle-pump connection pipes and 1 curved pipe
- A flask with pipe
- A curved collector connection, 1 connector 1 collector/tank connecting pipe, 3 collector/pump connecting pipes

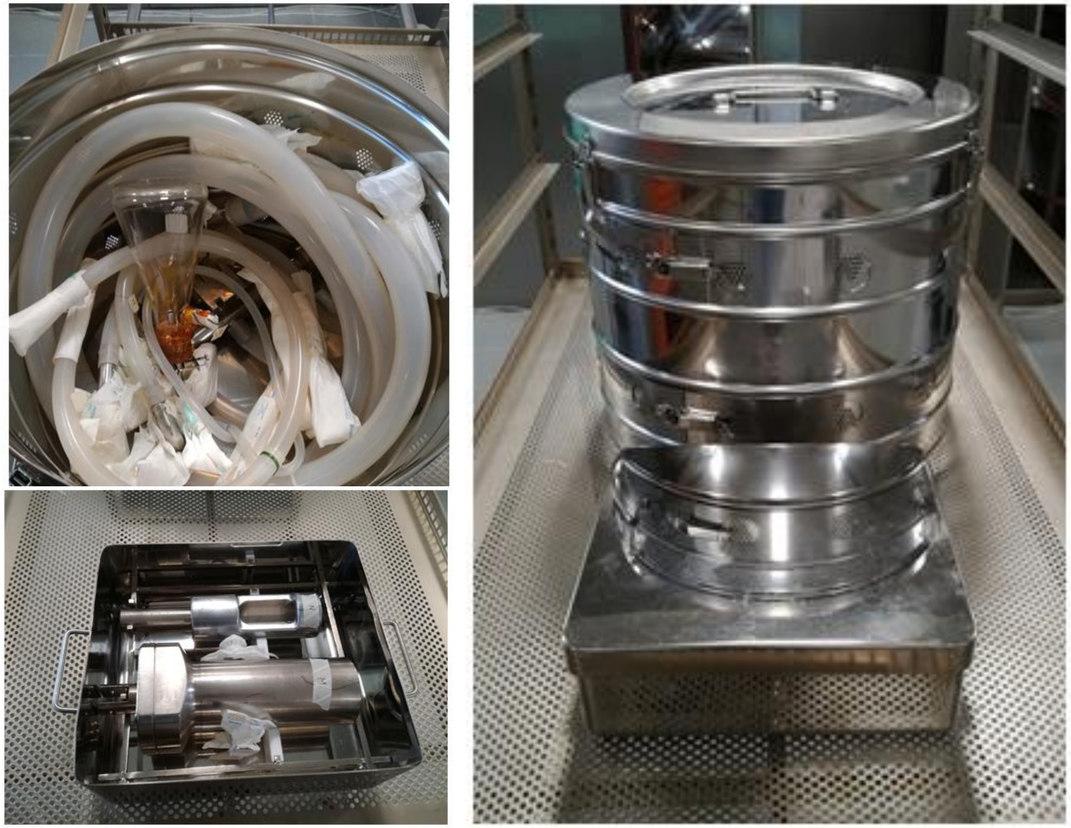
Autoclave type

Saturated steam sterilizer (FOF)

Customer request

Sterility and dryness of the load

Mixed Load

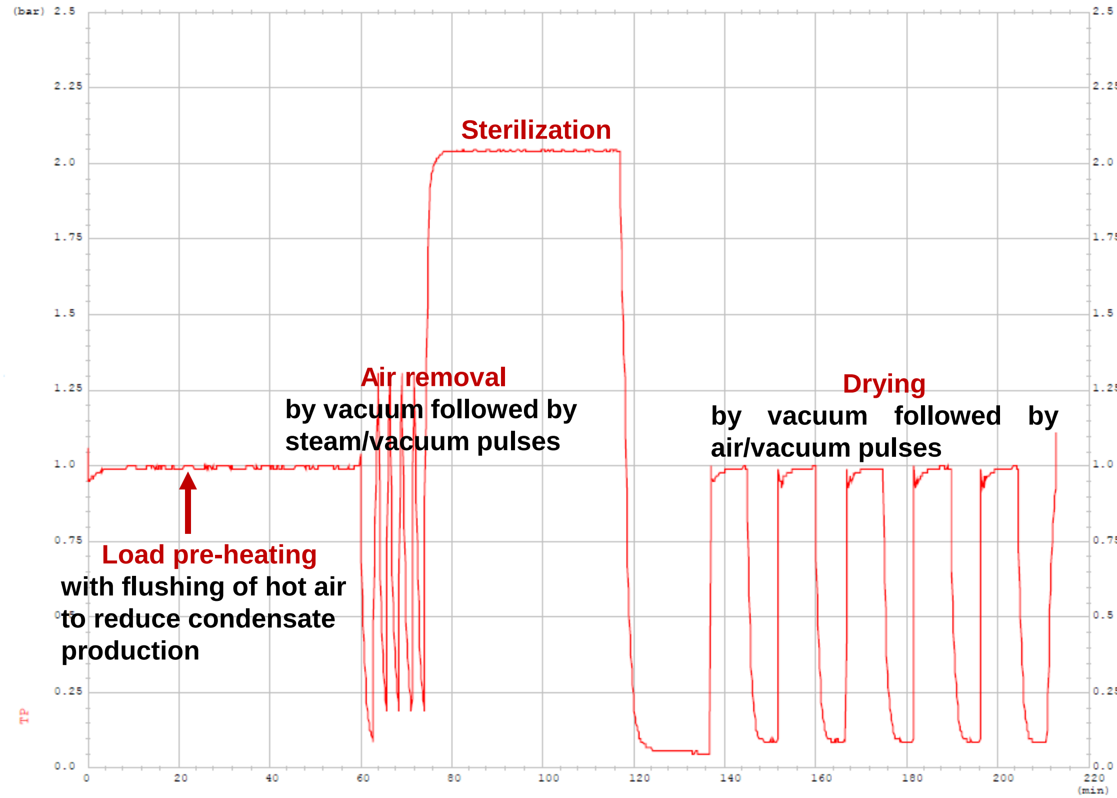


Mixed Load

PHASE LISTS

n. phase

1 PREPARE AUTOCLAVE
2 AIR TO CHAMBER
3 DEPRESSURIZE BY VACUUM PUMP
4 DYNAMIC STEAM RISING PULSE
5 FALLING PULSE
6 HEATING
7 STERILIZATION
8 DEPRESSURIZE BY VACUUM PUMP
9 TIMED VACUUM
10 RISING AIR PULSE
11 AIR PRESSURE HOLD
12 FALLING PULSE
13 TIMED VACUUM
14 RETURN TO ATMOSPHERIC PRESSURE
15 CYCLE END
16 EMERGENCY



Agenda

Introduction

Load types, Sterilization processes & Autoclaves

Saturated Steam Autoclave

Generality & cycle description

Counterpressure Autoclaves

Generality & cycle description

Counterpressure autoclaves

- Terminal treatment of solutions in **sealed containers**.
  Aim is to "**neutralize**" the effects of the **overpressure**

- Two types of **processes:**
  - **superheated water autoclave** (also, “water cascade”, or “water rain”)
  - **air-over-steam autoclave** (also, “steam & air”)

What appends when an aqueous solution in a sealed container is heated?

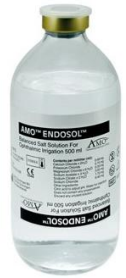
1. Water partially evaporates into the head space, but the *steam pressure* $\mathbf{P_v}$ in the head space depends only on the temperature, regardless to the head space volume, as *phase equilibria are not affected by mass transfer*

2. *Dissolved gases* partially leave the solution and generate a pressure $\mathbf{P_g}$ in the head space, that depends on temperature and chemical species

3. Gases (*air*) initially present in the head space expand, thus increasing their volume and/or their pressure $\mathbf{P_a}$, that depends on gas mass, temperature and head space volume

4. The liquid phase increases its volume (thermal expansion of the liquid is practically not containable)
→ *This tends to reduce the head space and increase the pressure*

5. The overall capacity of the container increases thanks to the thermal expansion of its material (the thermal expansion is quite different for plastics, glass and metals).
→ *This tends to increase the head space and reduce the pressure*



$$P_v + P_g + P_a = \mathbf{P_h}$$

is the **total pressure in the head space**

Overpressure inside sealed container

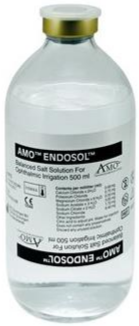
The total pressure (P) generated inside the sealed container at the temperature T (ex. 121° C) is equal to:

$$P_{(T)} = Pv_{(T)} + Pa_{(T)}$$

Where:

Pv= Pressure of the water vapor

Pa = Pressure of the air



- Air initially present in the head space.
- Dissolved gases that come out of the solution.
- Reduction of the head space due to the thermal expansion of the liquid.

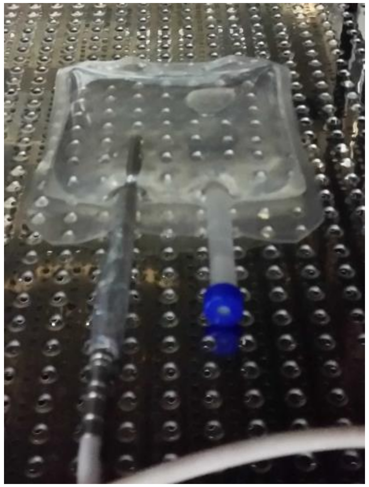
Example: Calculation of the counterpressure required at 121ºC

P_v → It's a well-known value (121ºC → 2,05 bar abs)

P_a → It's calculated based on the temperature of the liquid

The **total pressure** inside the chamber (counterpressure) is automatically **controlled and adjusted** according to the **temperature of the solution.**

The sterilization process is «driven» by a **temperature probe (product probe) directly placed inside the container with the liquid.**



Counterpressure Autoclaves

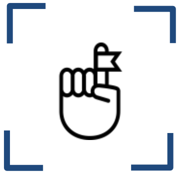
Superheated water autoclave (FOW)
Fedegari Orizzontale Water/ Fedegari Horizontal Water

- **“Water cascade” sterilizers**
- **Counterpressure sterilization** through superheated water

Steam-air mixture autoclave (FOA)
Fedegari Orizzontale Aria/ Fedegari Horizontal Air

- **Steam air mixture** sterilizers able to perform counterpressure sterilization

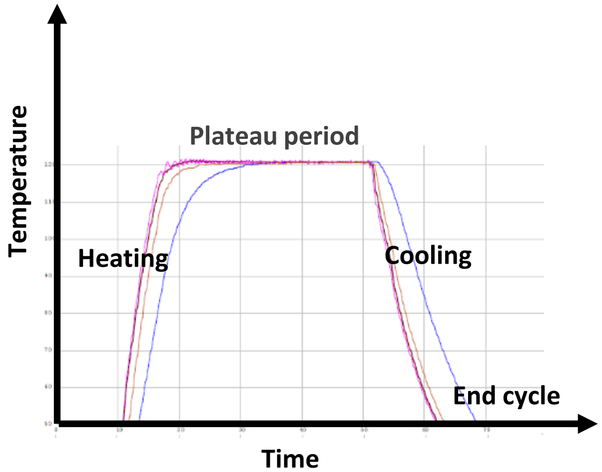
> 100ml



- At beginning the **air in the chamber is not removed**
- Suitable for treating loads in containers that may be **deformed** due to the difference in pressure between the chamber and the container itself.

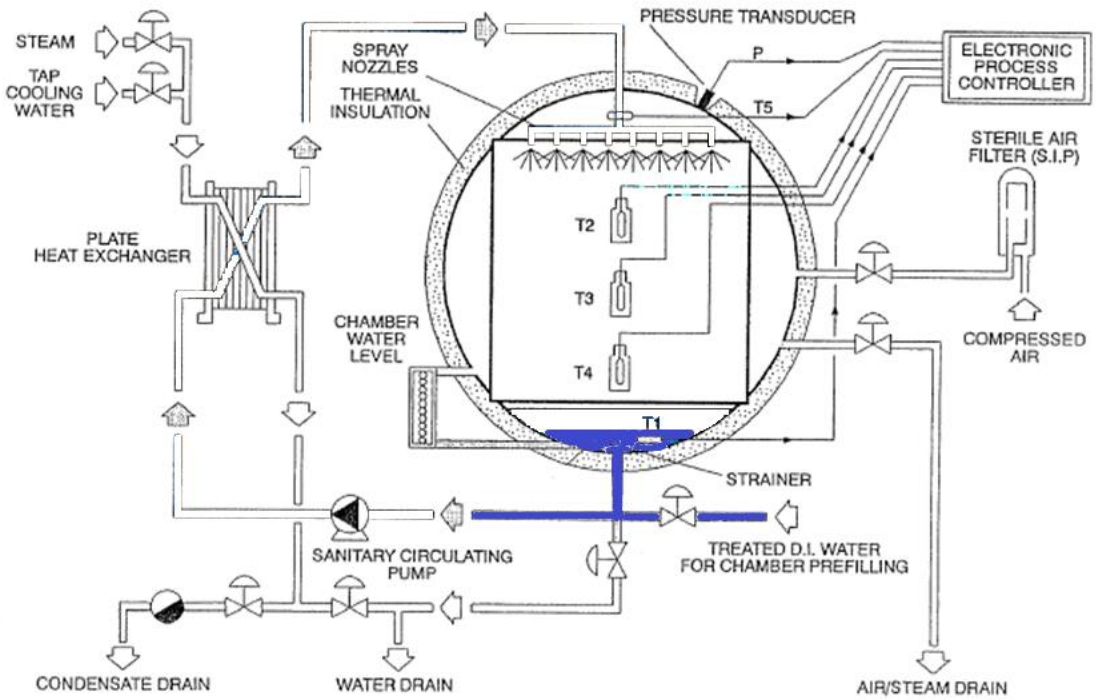
Superheated water autoclave (FOW)

Process phases

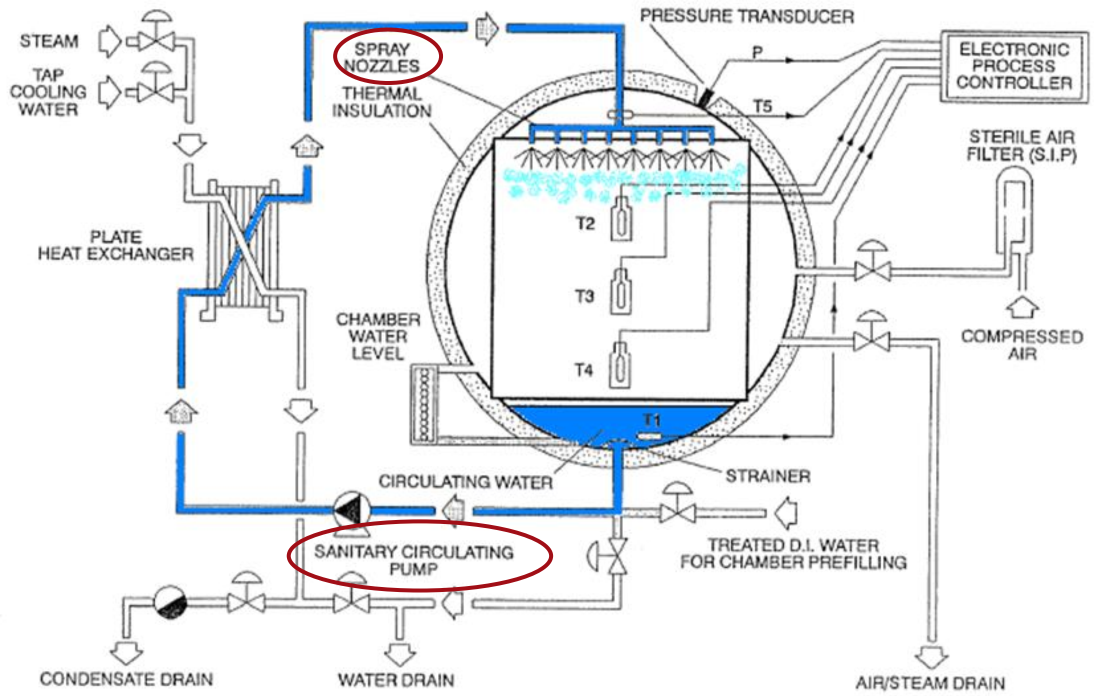


1. Chamber water filling
2. Water circulation
3. Heating & Sterilization
4. Cooling
5. Chamber drain
6. Atm pressure balance

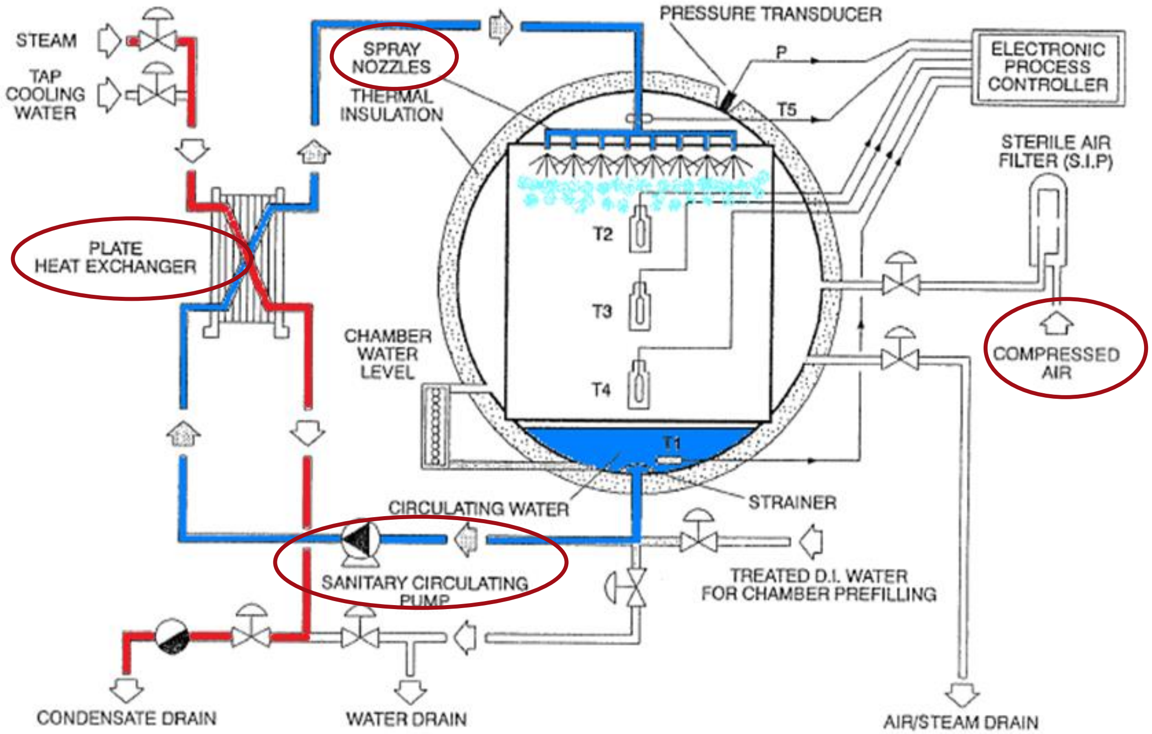
Phase 1: Chamber water filling



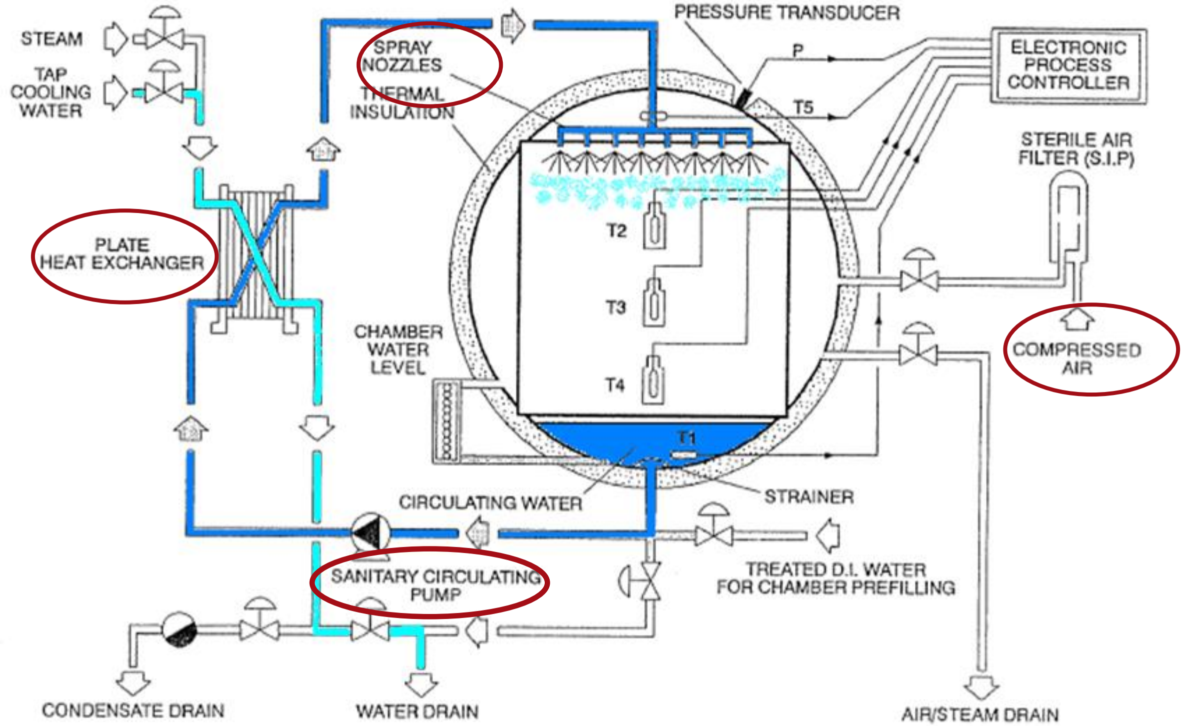
Phase 2: Water circulation



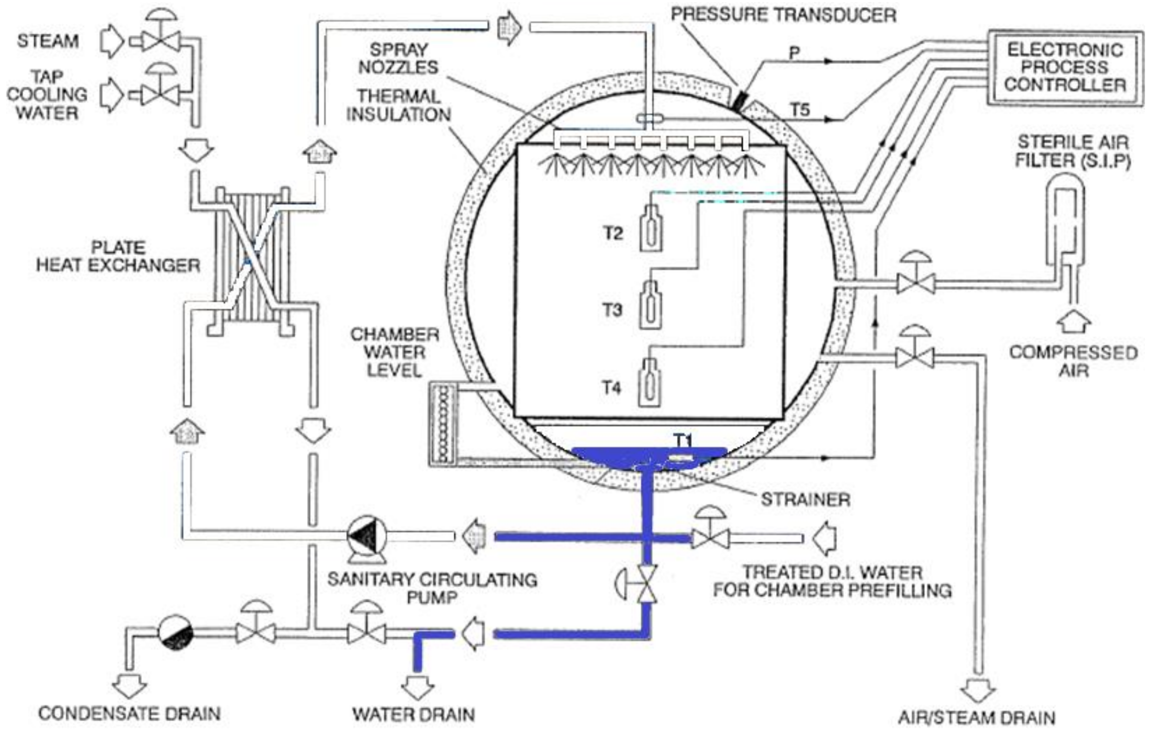
Phase 3: Heating & Sterilization



Phase 4: Cooling



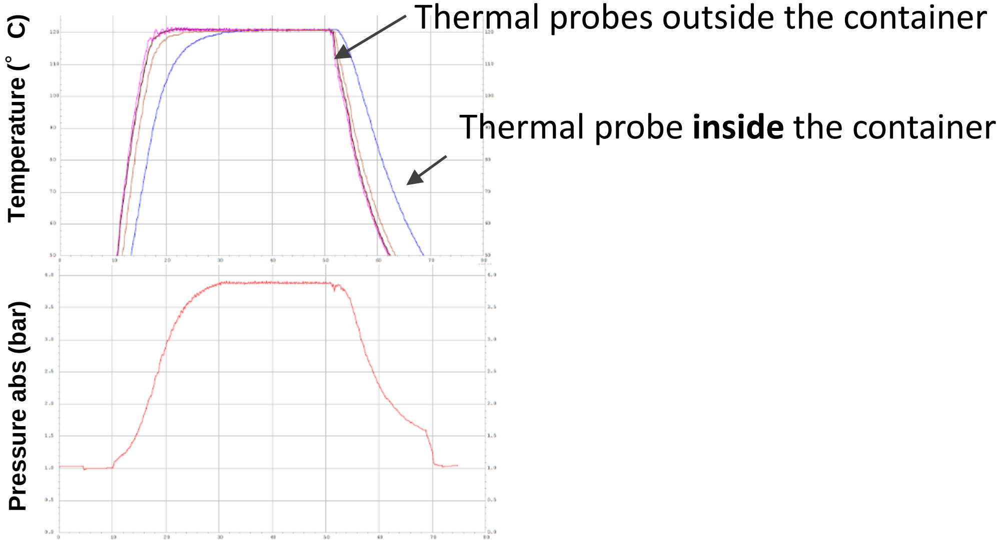
Phase 5: Chamber drain



Sterilization cycle description

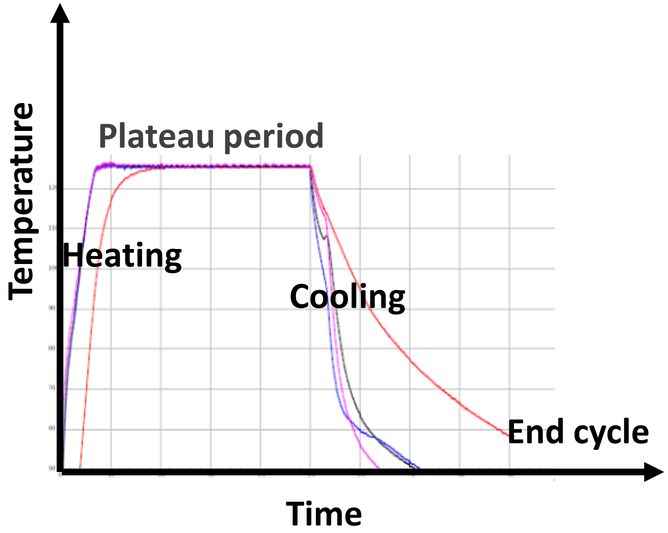
- The **control of the process** is based on the temperature
- **Chamber pressure** is independent from the temperature
- During all stages the **counterpressure** is controlled

Thermal & Pressure profile



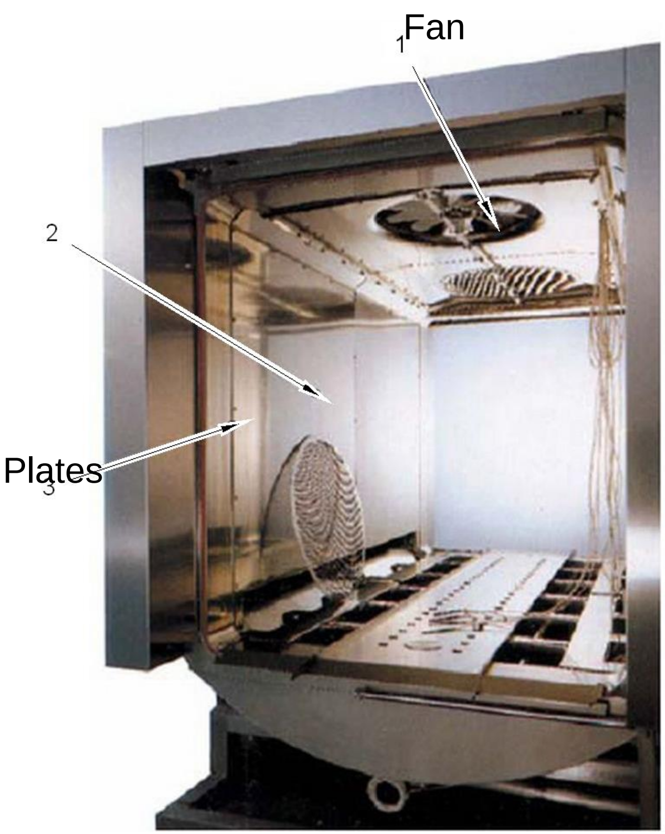
Steam- Air mixture autoclave (FOA)

Process phases



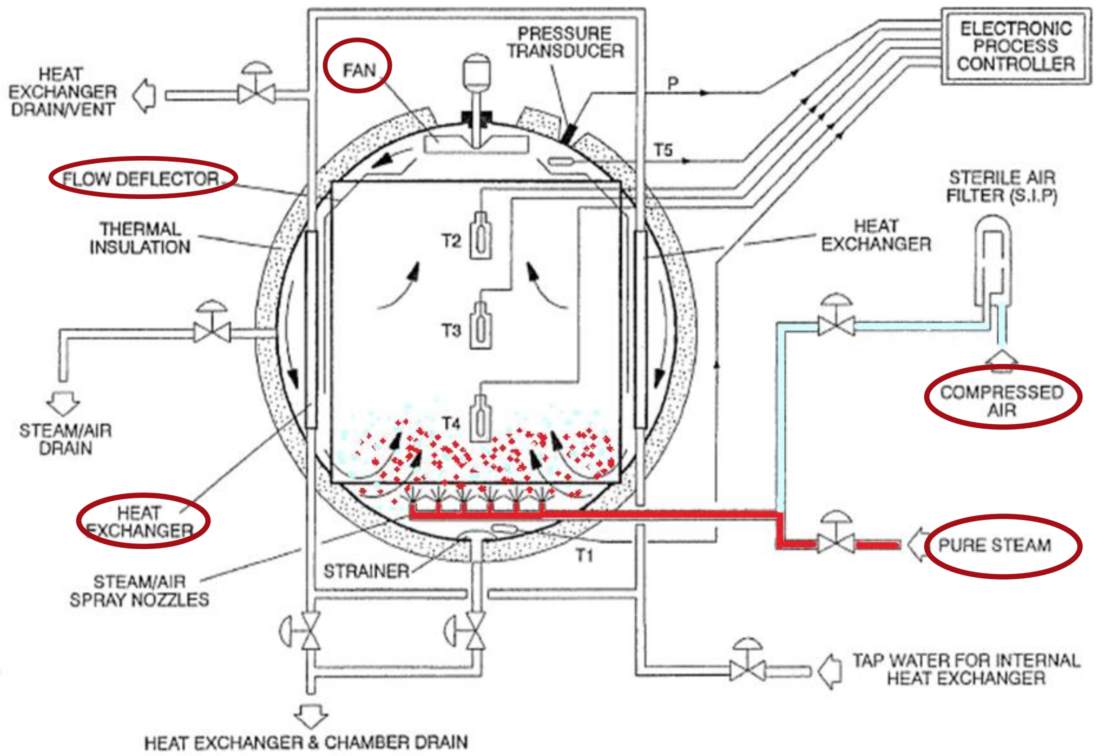
1. Heating & Sterilization
2. Cooling
3. Atm pressure balance

Steam- Air mixture autoclave (FOA)

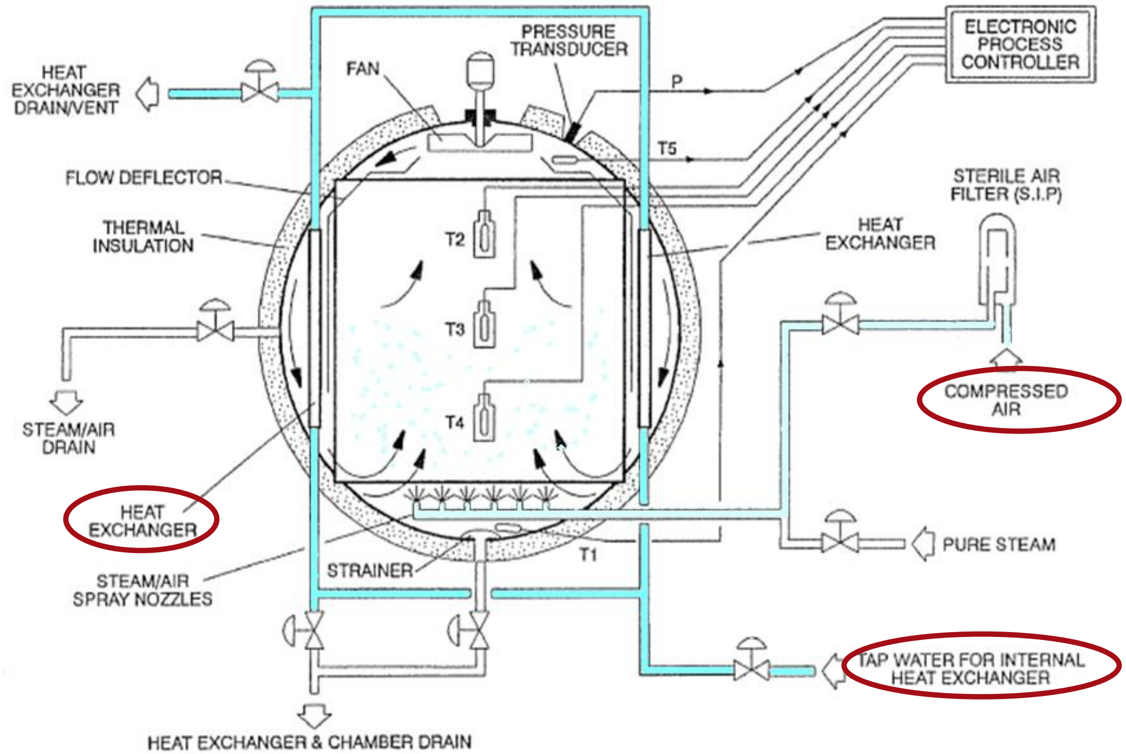


- At beginning the **air in the chamber is not removed**
- **Fan**(s) and **flow deflectors** allow to homogenize and distribute the steam-air mixture

Phase 1: Heating & Sterilization

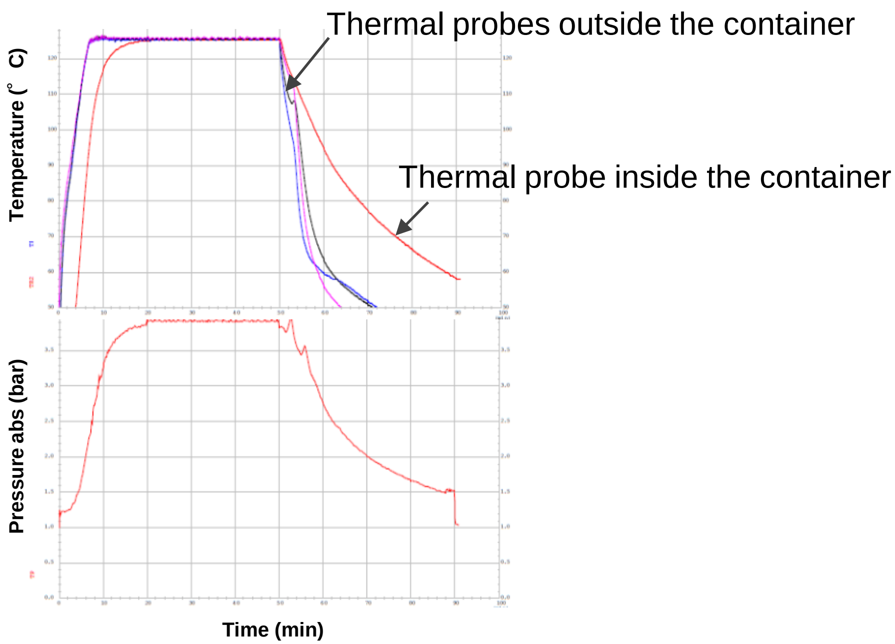


Phase 2: Cooling



Thermal & pressure profiles

- The **control of the process** is based on the temperature
- **Chamber pressure** is independent from the temperature
- During all stages the **counterpressure** is controlled



Note: the FOA and FOW charts are similar because they are both counterpressure machines.

Air-over-steam autoclaves: Steam requirements

In air-over-steam autoclaves:

- there is contact between steam and product, just as in saturated steam autoclaves
- steam heats only or heats and sterilizes depending on the type of load (containers or "difficult" products: blood-bag systems, dialysis filters, containers in blister)
- if the steam heats and sterilizes, the same steam quality requirements do apply, as in the case of saturated steam sterilization, but
- a further difficulty derives from the independence of pressure and temperature due to the presence of air; the ratio of partial pressures of steam and air is usually between 3 and 1.1 (most commonly about 1.5)

Comparison

FOW

- \+ Easy controlled modulated heating and modulated cooling
- \+ Shorter process duration
- \+ No appreciable consumption of clean steam (used only for filter sterilization)
- – Product is unloaded wet
- – Higher water consumption (for initial filling)
- – Higher energy consumption (to heat the circulation water)
- – Blushing phenomenon (i.e. whitening of the PVC due to water absorption)

vs

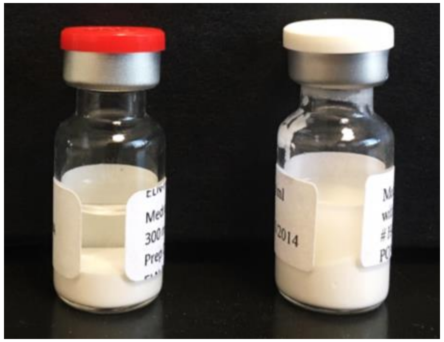
FOA

- – Controlled modulated heating but not possible modulated cooling
- – Longer process duration (mainly because of indirect cooling)
- – Low unloading temperatures require much time
- – Modulated cooling impossible; (but modulated heating possible)
- – Consumption of clean steam
- \+ Lower energy consumption
- \+ Product could be easily unloaded dry
- \+ No PW/UPW/WFI water consumption
- \+ Blushing phenomenon very rare

Case studies

- Suspension in a glass vial
- Plastic bottles
- Liquids in plastic sealed containers

Suspension in glass vials



Load description

- 1 ml suspension
- 3 ml glass vials sealed with rubbers stoppers and flip-off caps
- 2 different preparations

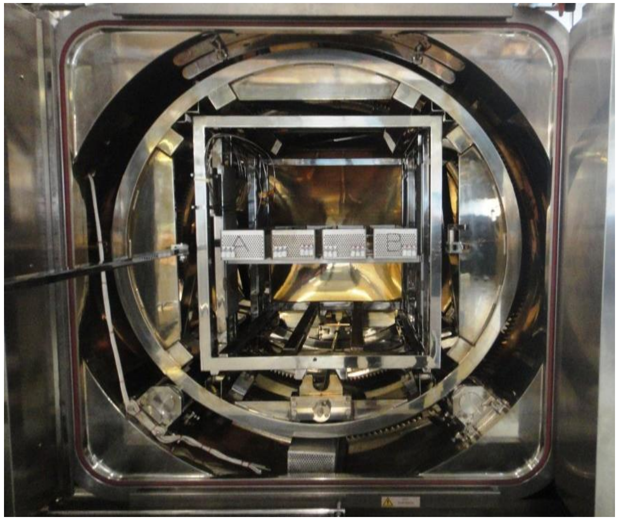
Customer request

- Minimal thermal treatment at 121°C in a superheated water autoclave
- Maintain the physical stability of the suspension after the treatment
- Evaluate if the cake formation is hard to re-suspend and the time taken to re-suspend it

Suspension in glass vials

Process strategy

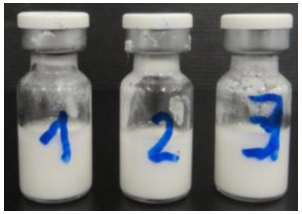
- Superheated water autoclave
- Rotating basket and different rotating speed



Suspension in glass vials

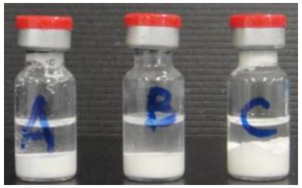
Result

White samples



- The samples were suspended.
- All the samples had the same behaviour independently from the position.
- All the samples lost their paper label.
- All the samples resulted re-suspended in about 5’’ of manually shaking.

Red samples



- The two phases in the samples were still separated.
- All the samples had the same behaviour independently from the position.
- All the samples did not result completely suspended in 30’’ of manually shaking.

Plastic bottles

Load description

- Plastic bottles
- Material: Polypropylene
- Size: 250 ml
- Content: Water/placebo

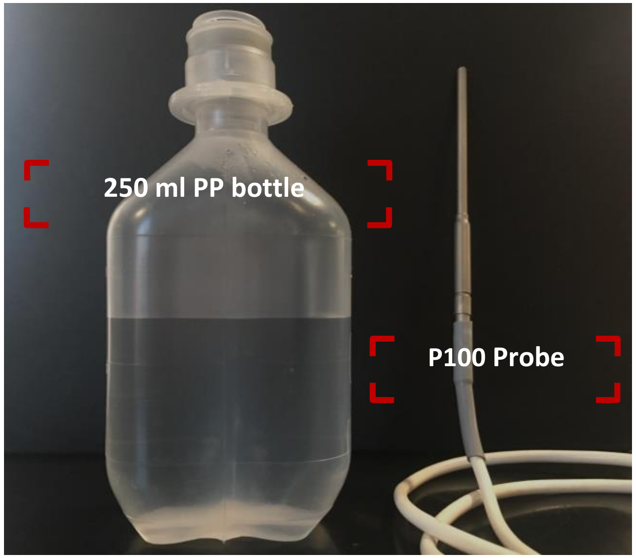
Customer request

- No deformation
- Sterilization at 121°C for 20’ in a superheated water autoclave

Plastic bottles

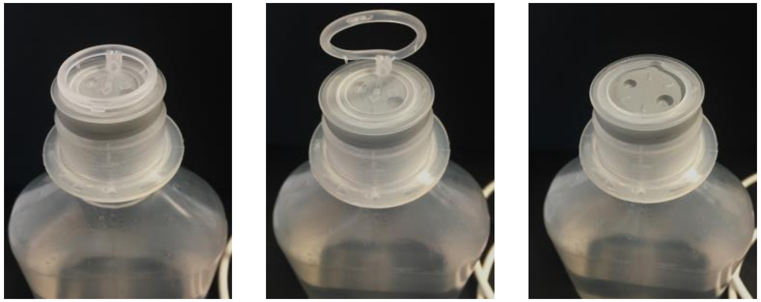
Process control

The sterilization process is «driven» by a temperature probe (P100 product probe) directly placed inside the container with the liquid.

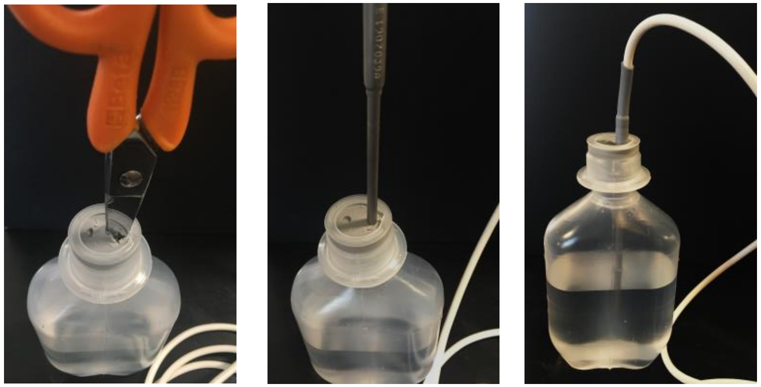


Plastic bottles

Step 1
Remove the protective ring



Step 2
Pierce the silicon stopper and insert the P100 thermal probe

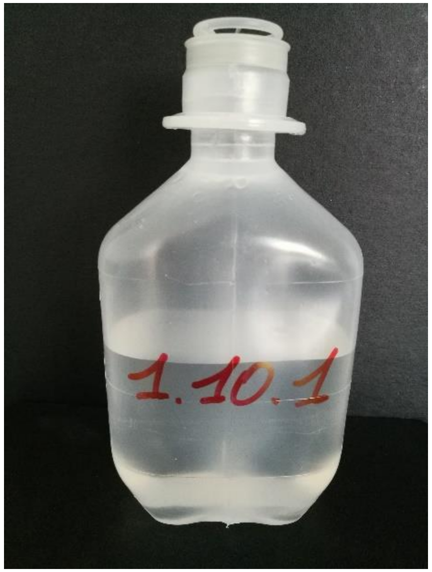


Step 3
Place the load and the bottle with the thermal probe in the FOW chamber

Plastic bottles

Result

No deformation



Liquids in plastic sealed containers

What happen if…

- Temperature is too high?
- The applied counterpressure is not well adjusted?

Container deformation!!

Liquids in plastic sealed containers

Load: 500 mL plastic bags

Autoclave type: FOA

Challenge: bag integrity

Result

Intact bag

Thank you for your attention!