Moist Heat Sterilization

Load types and processes - Autoclave selection

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Counterpressure Process

General Concepts

Counterpressure autoclaves

Steam-air mixture
‘Counter pressure’ autoclave

Superheated water
‘Counter pressure’ autoclave

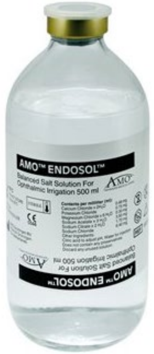
Counterpressure autoclaves: Liquid Loads

PHARMA

INDUSTRIES

Counterpressure autoclaves: Liquid Loads

What happens when an aqueous solution in a sealed container* is heated



1. Water evaporated in the head space
2. Dissolved gases leave the solution
3. Gases (air) initially present in the head space increases their volume
4. The liquid phase increases its volume (thermal expansion of the liquid)
5. The container capacity increases as a result fo the thermal expansion of it's material (the thermal expansion is different between glass and plastic containers)

↓ Head space volume

↑ Head space volume

*Sealed= hermetically closed

Counterpressure autoclaves: Liquid Loads

What happens when an aqueous solution in a sealed container* is heated

The **pressure** in the head space (sealed container) is the sum of: -

1. Vapour pressure of the liquid solution. It depends only on the temperature (one-to-one correspondence between P/T in the vapor and liquid equilibria) and it's indipendent from the quantity of product.
2. Pressure of the gases escaped from the liquid solution.
3. Pressure of the gas initially present in the head space. Its pressure will rise proportionally to the absolute temperature (and number of moles) and with the reduction of the volume of the head space (perfect-gases-law: $P=nRT/V$)

The head space volume will tend to decrease because of the expansion of the liquid volume and the pressure will consequently increase

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Counterpressure autoclaves: Liquid Loads

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

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

P_v is a well-known **value (**121° C → 2.05 bar abs**)**

P_a is calculated based on the temperature of the liquid

Counterpressure autoclaves: Liquid Loads

Bottle partially filled with water solution at 20° C and 1bara subjected to a saturated steam sterilization at 121° C

In the chamber: 2.05 bar
Saturated steam pressure

Saturated steam pressure = 2.05 bar

\+

Air pressure = 1.34 bar

Total pressure in the bottle ≅ 3.4 bar

$\Delta P \cong 1.4$ bar → 1.4 kg/cm

Counterpressure autoclaves: Liquid Loads

How do we calculate the total pressure inside the container ($P_{(T)} = Pa_{(T)} + Pv_{(T)}$) (in order to define the required counterpressure in the chamber) ?

$Pa_{(T)}$ (Air Pressure at "T" Temperature) =

$$= K * 0.971 * (T + 273.15) / (30 + 273.15)$$

Assuming:

K = "Pa" Correction Coefficient (a program parameter)

It corresponds to the atmospheric pressure

0.971 bar = Initial air pressure in the container when sealed
= $P - Pv_{(30\,°C)}$ = 1.013 - 0.042

T (°C) = Temperature of the liquid in the container during the process

30 °C = Assumed initial temperature of the liquid in the container

273.15 = "zero absolute" offset, to convert temperature values from °C to K

Counterpressure autoclaves: Liquid Loads

Example: calculation of the counterpressure (P) required at 121°C using a typical k value= 1.08

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

1) Pa $= 1.08 * 0.971 * (121 + 273.15) / (30 + 273.15) = \quad =$ 1.36 bar

2) Pv = 2.05 bar

3) $P_{(T)}$ = 1.36 bar + 2.05 bar = 3.41 bar

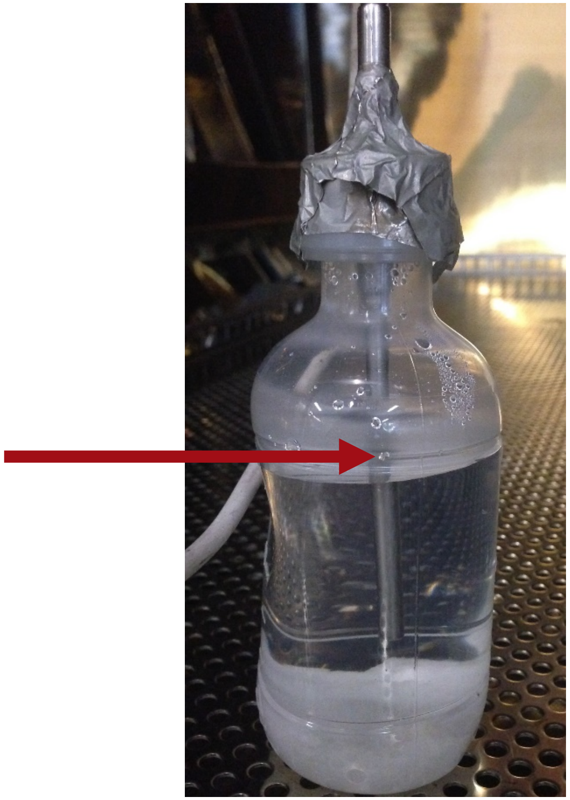
Counterpressure autoclaves: Liquid Loads

The total pressure inside the chamber (counterpressure) is automatically controlled and adjusted according to:

- ✓ **Temperature of the solution**
- ✓ **Container features** (ex. rigid or deformable material)

Counterpressure autoclaves: Liquid Loads

The sterilization process is typically «driven» by a temperature probe ’load probe’ directly placed inside the container with the liquid



Counterpressure autoclaves: Liquid Loads

An aqueous solution increases its volume about 6% when heated from ambient temperature to 121°C.

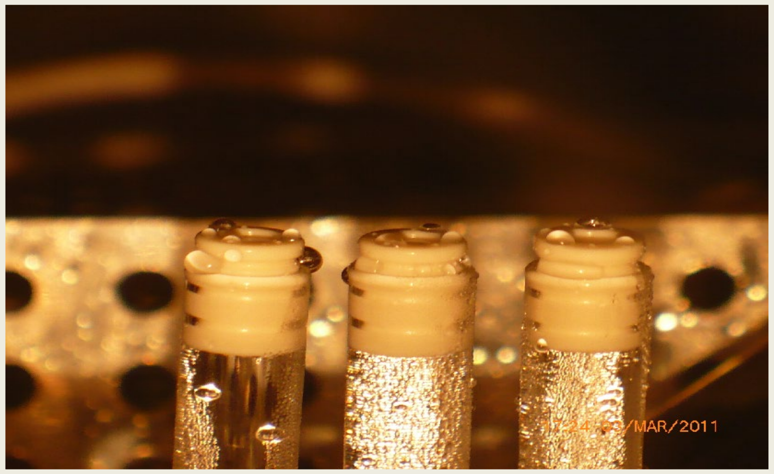
↓

Thermal expansion of the water becomes important if the head space is lower than 10-15% of the volume of the container

Counterpressure autoclaves: Liquid Loads

There is no practical mean to prevent the thermal expansion of liquids during sterilization.

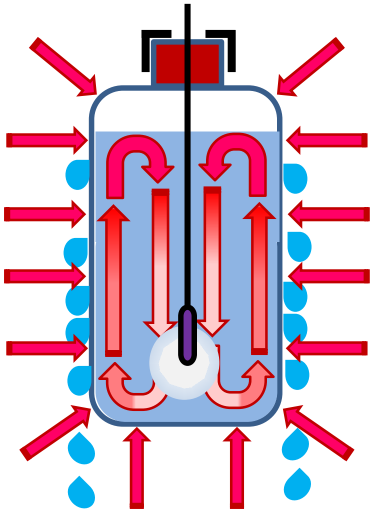
The pressure required to reduce the volume by 6% of a liquid like water would be very large: thousands of bars !!!

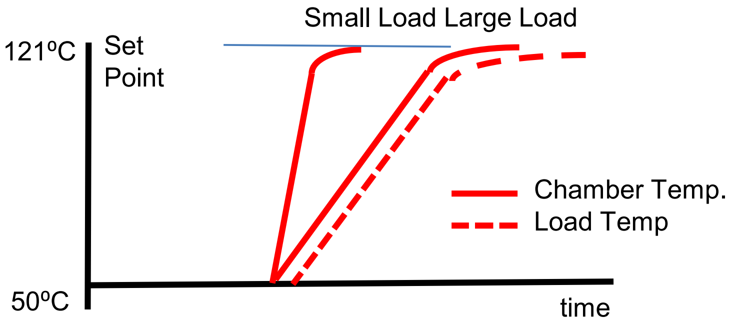


Counterpressure autoclaves: Liquid Loads

The container cannot be totally filled with the liquid, a certain head space is always necessary

Counterpressure autoclaves: Liquid Loads





Steam condenses on the container surface.
Latent heat is released, heating the container and the
fluid, condense runs to drain, replaced by new steam
Fluid contents heat through by thermo-recirculation

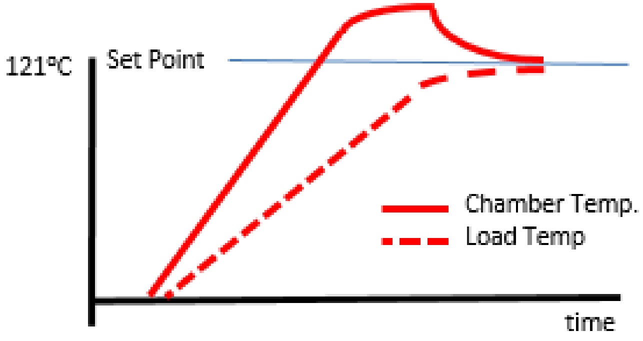
For validation and control, coolest point is central, typically 10mm up from the base.

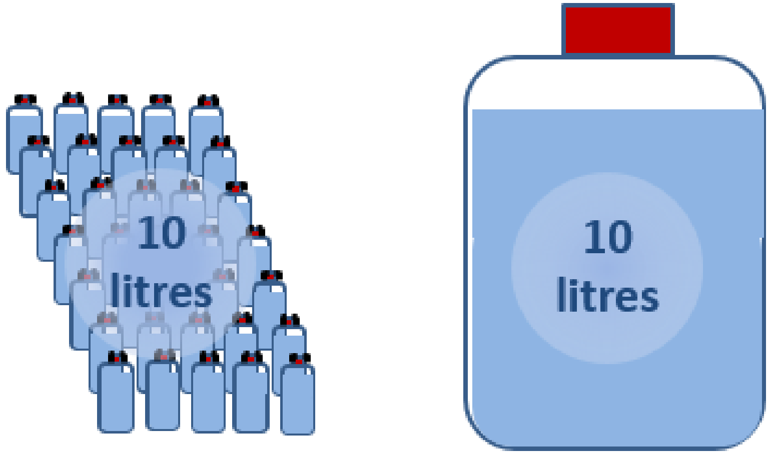
Chamber heats rapidly if the load is small, however the fluid contents take time to heat through

Chamber heats more slowly if the load is large, but heating of the contents may be much slower

The validated cycle must represent the size of the load as well as the configuration of container & fluid.

Counterpressure autoclaves: Liquid Loads

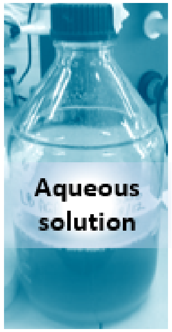




Fast heat penetration

Slow, even hours

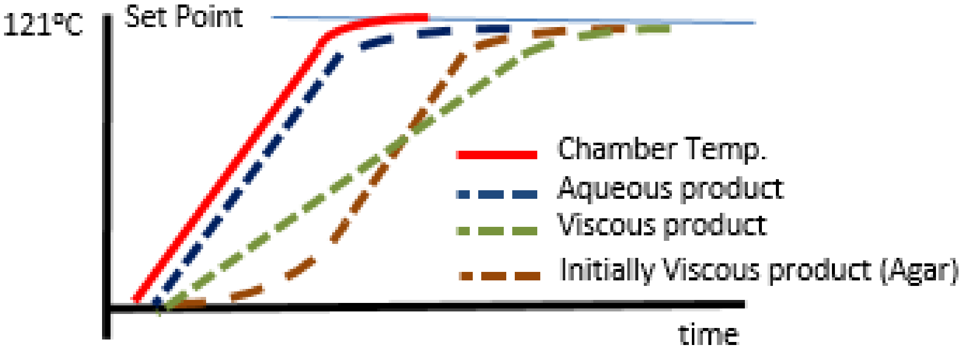
Counterpressure autoclaves: Liquid Loads



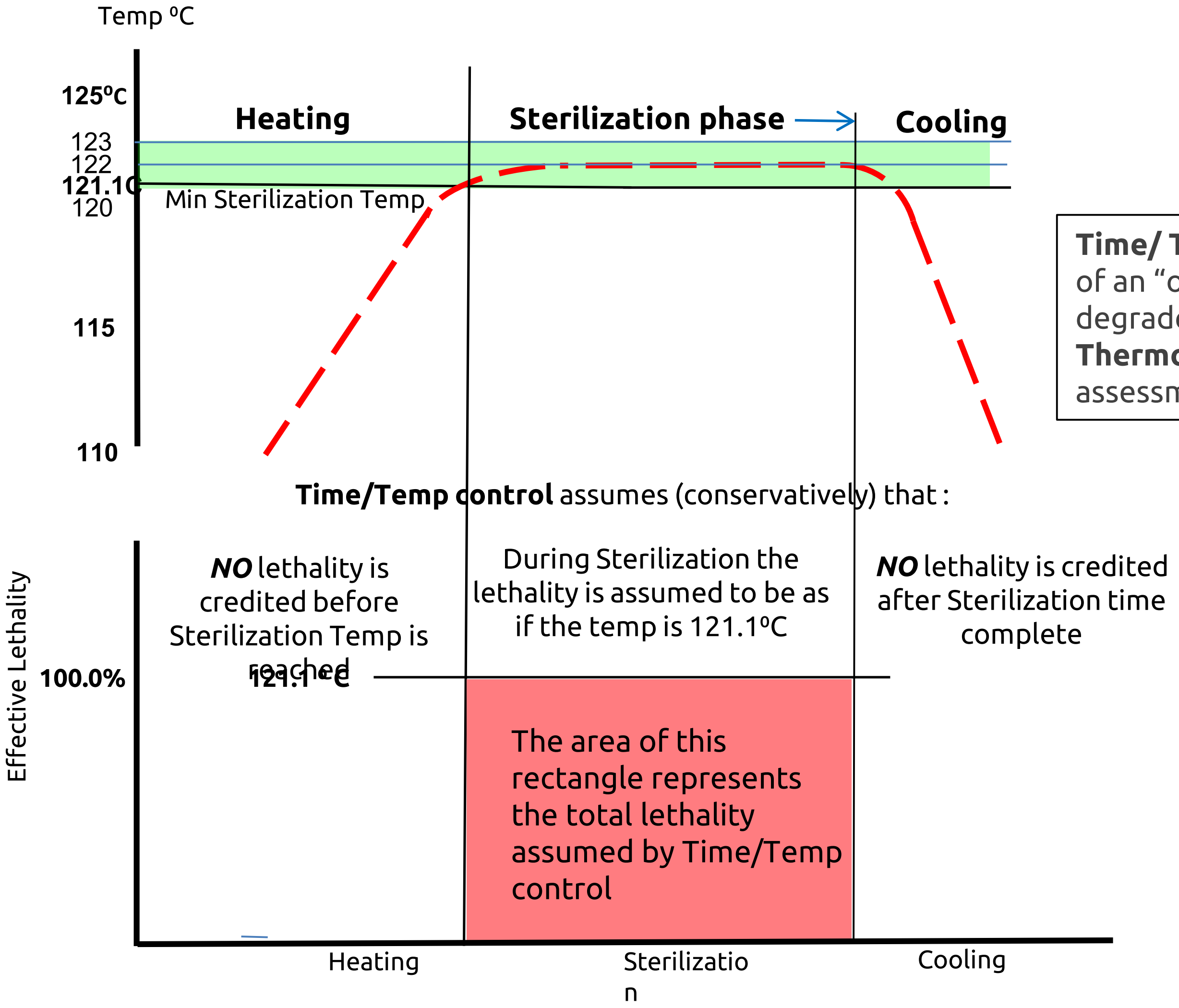
Low viscosity.
Good thermo-cycling.
Fast heat penetration

High viscosity
Slow thermo-cycling.
Slow heat penetration

High viscosity/slow thermo-cycling at first
Heat penetration better when hot



Counterpressure autoclaves: Liquid Loads



Time/Temp control assumes (conservatively) that :

NO lethality is credited before Sterilization Temp is reached

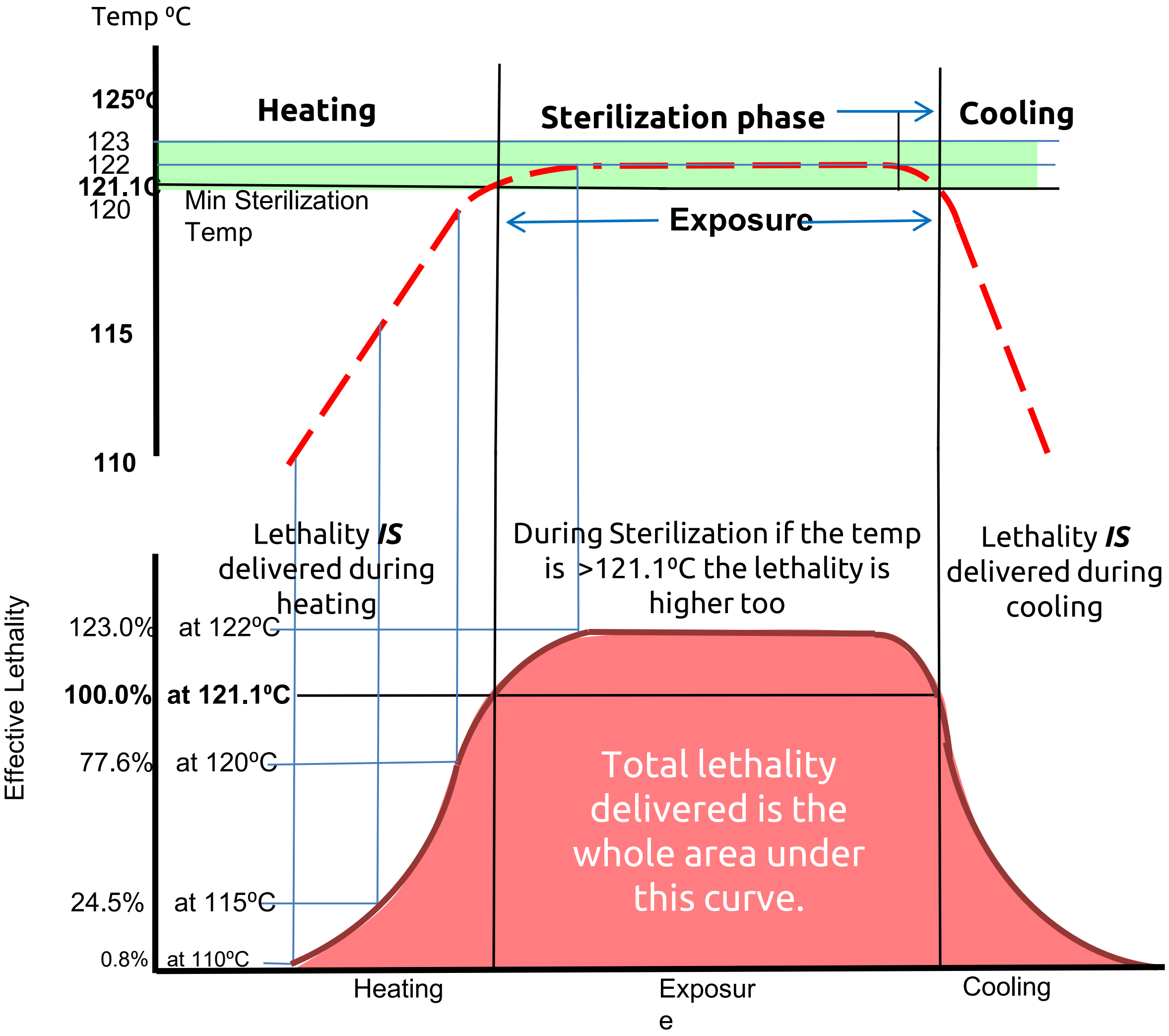
During Sterilization the lethality is assumed to be as if the temp is 121.1°C

NO lethality is credited after Sterilization time complete

The area of this rectangle represents the total lethality assumed by Time/Temp control

Time/ Temperature Control is used as part of an "overkill approach" where loads are not degraded by temperature.
Thermolabile fluids need a more accurate assessment of lethality delivered.

Counterpressure autoclaves: Liquid Loads

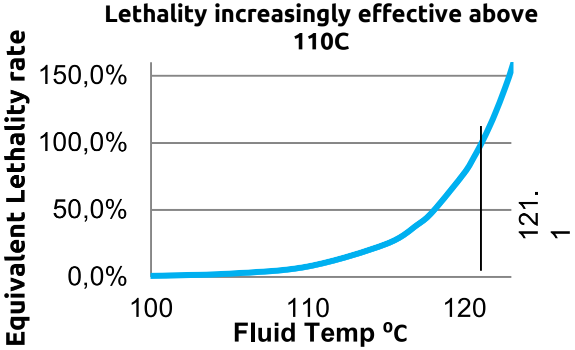


Lethality *IS* delivered during heating

During Sterilization if the temp is >121.1°C the lethality is higher too

Lethality *IS* delivered during cooling

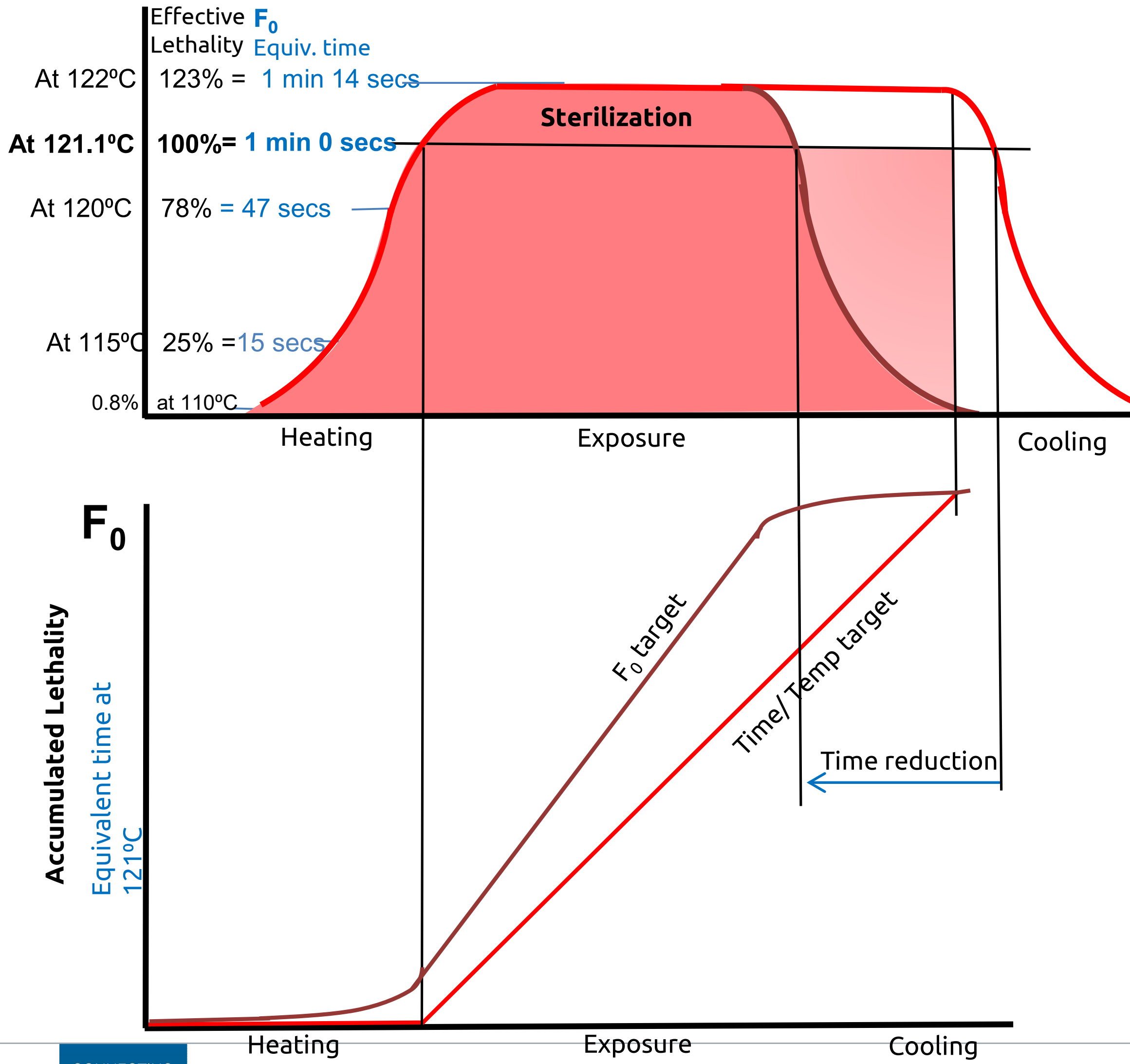
Total lethality delivered is the whole area under this curve.

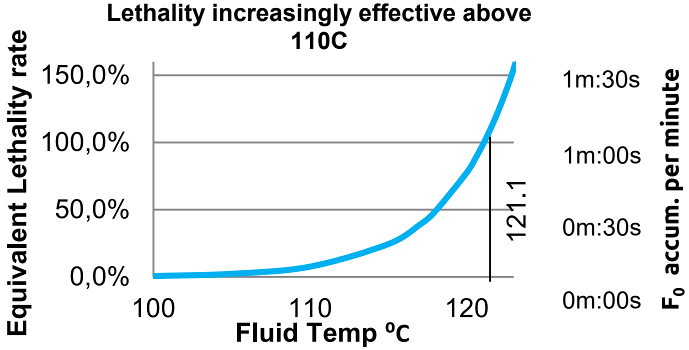


Equivalent Lethality at temp T = $10^{(T-121.1)/10}$
(Where z = 10)

There is more lethality delivered during a Sterilization cycle than assumed by Time/Temperature measures.

Counterpressure autoclaves: Liquid Loads

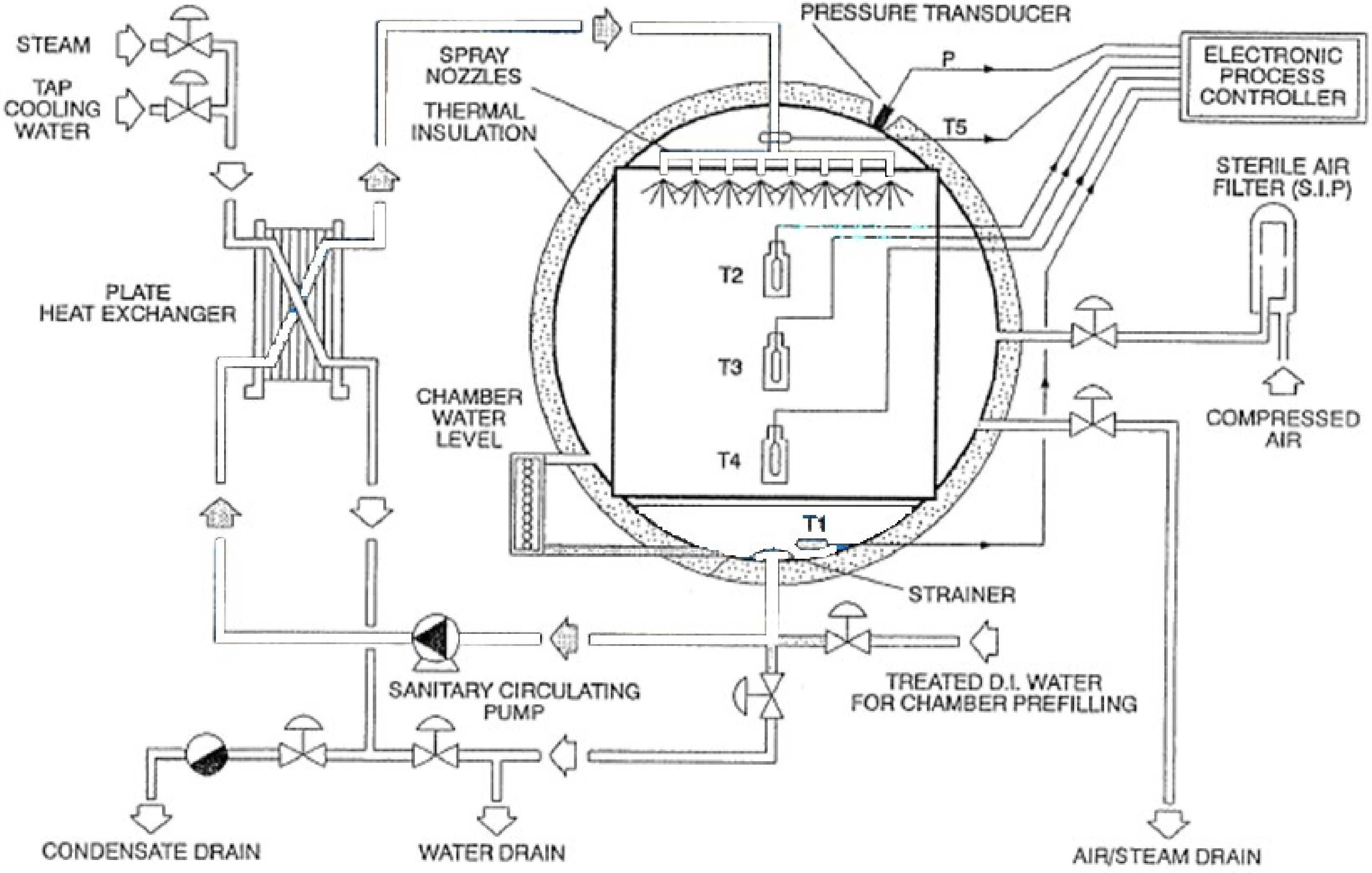




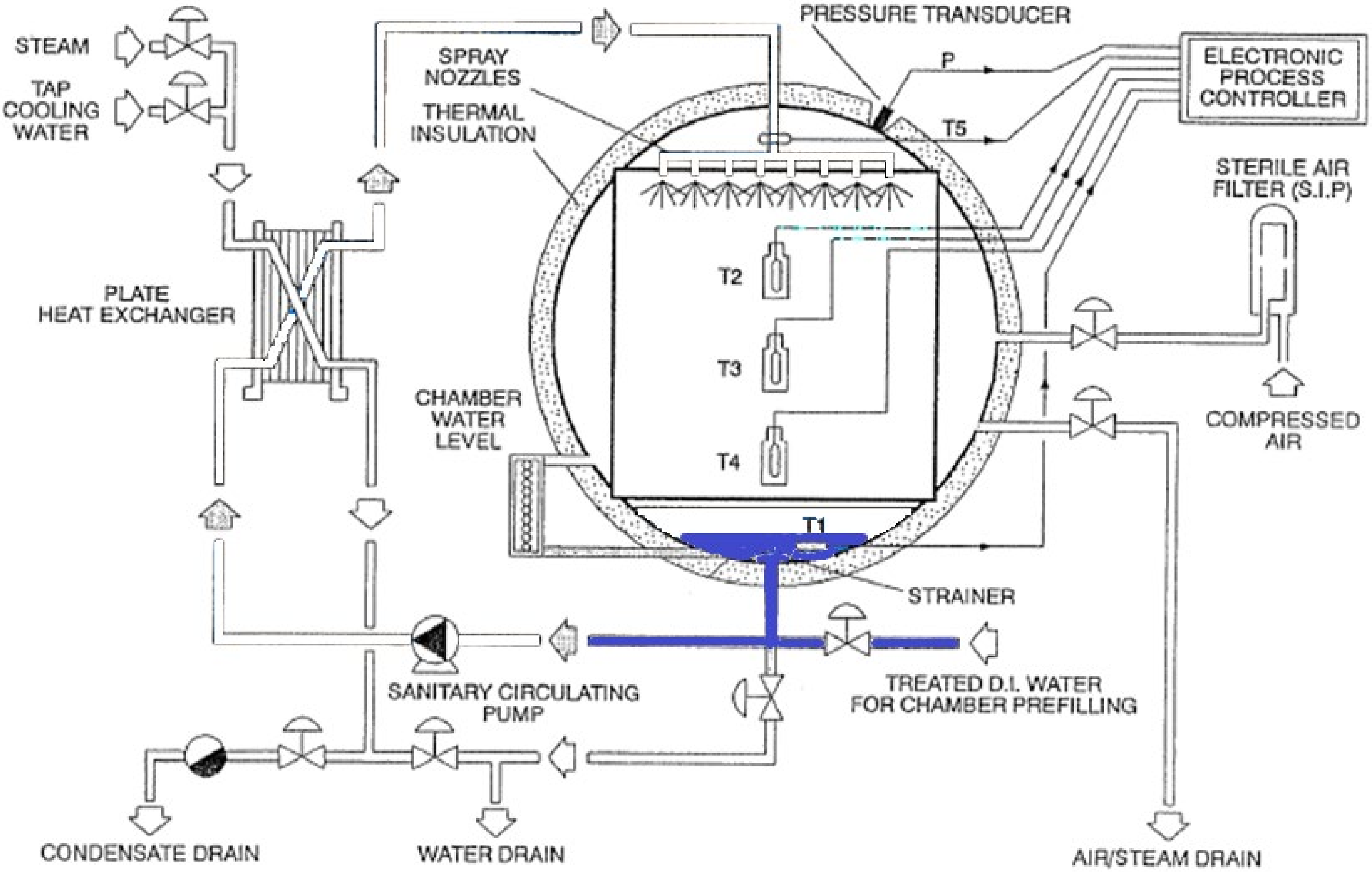
Targeting accumulated F_0 rather than Time at Temp. can be used to shorten the load exposure to high temperatures

Superheated water autoclave

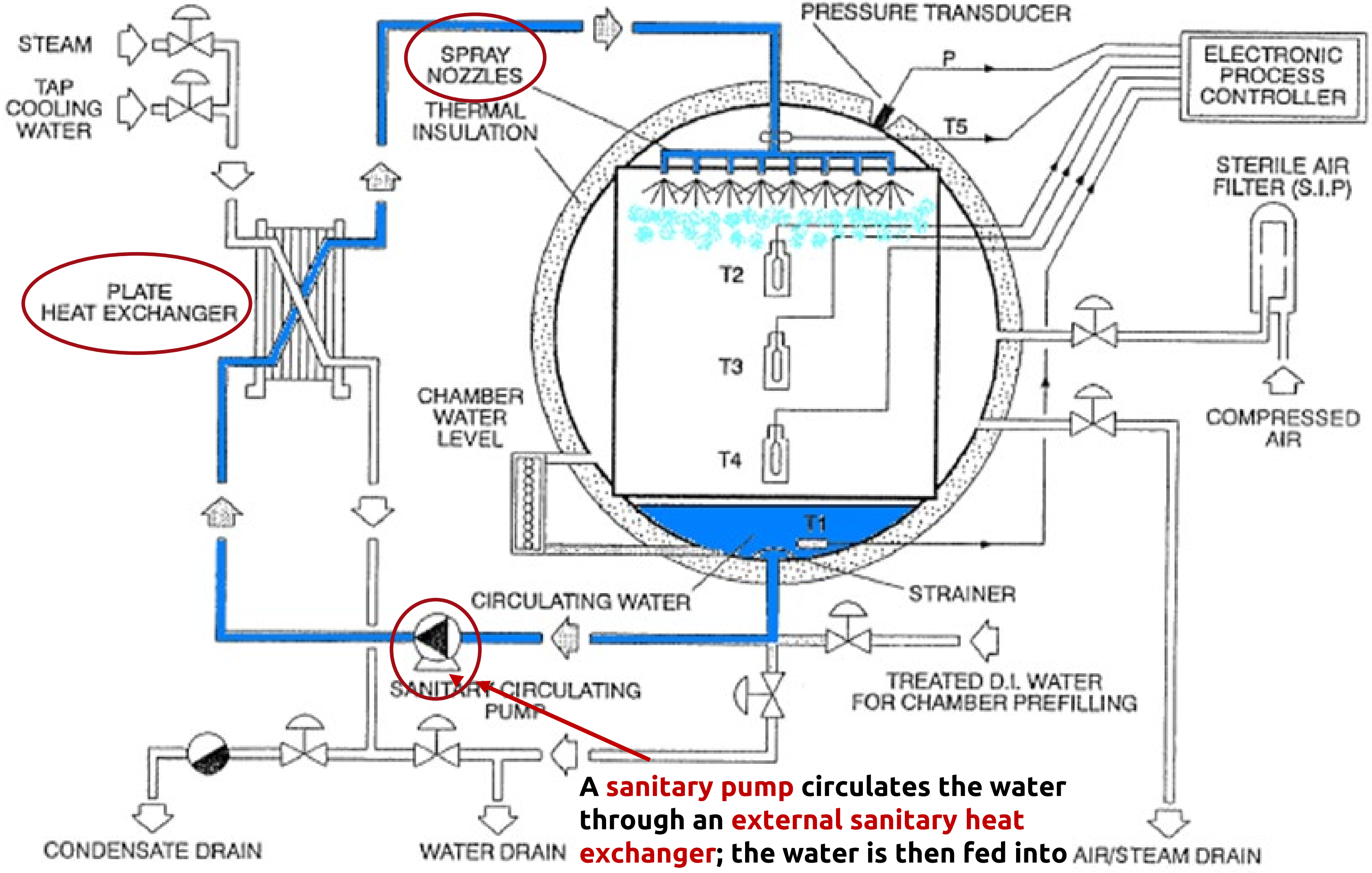
Superheated water autoclave – schematic



Chamber filled by water

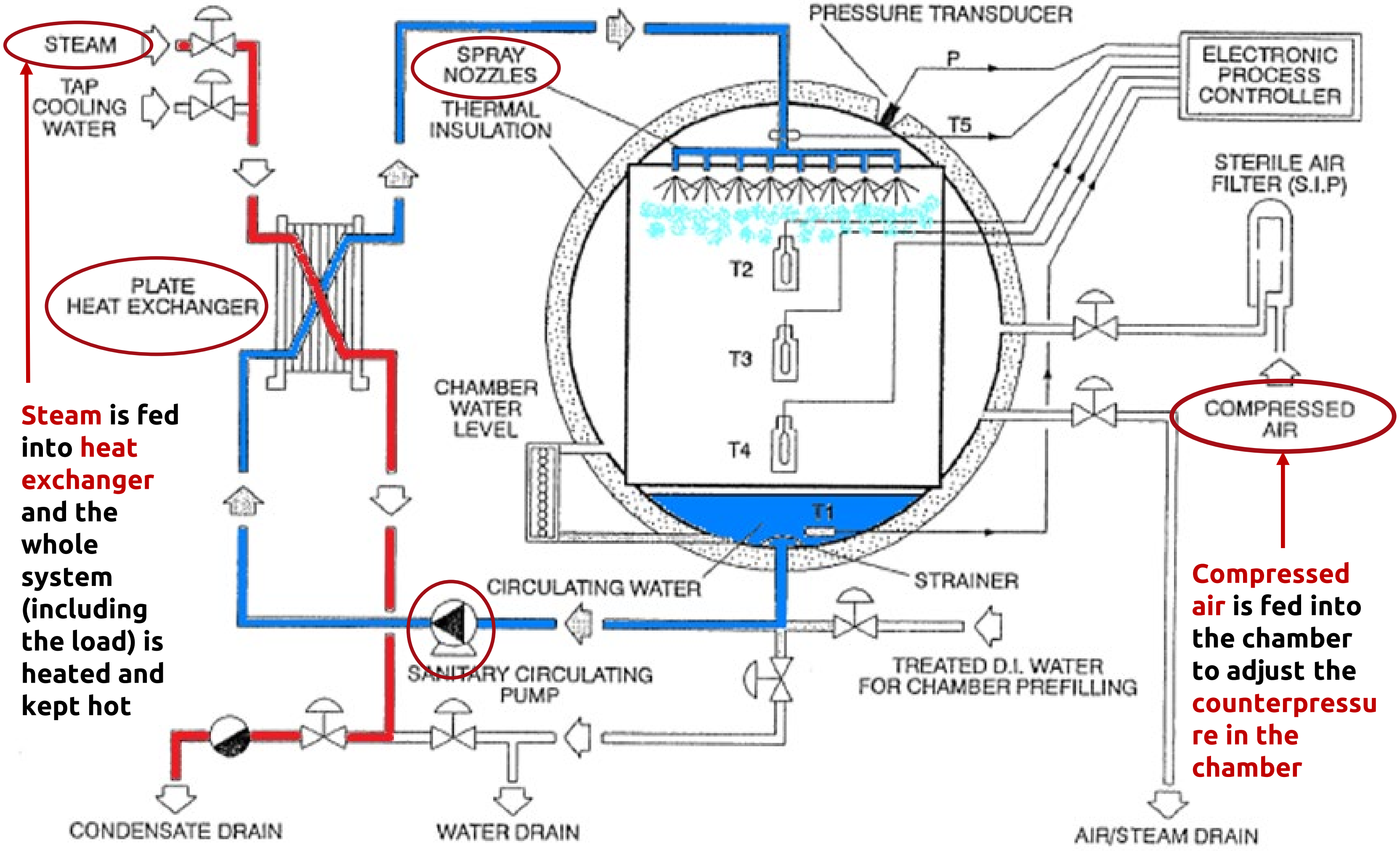


Water circulation



A **sanitary pump** circulates the water through an **external sanitary heat exchanger**; the water is then fed into the upper part of the chamber through a **sparger**

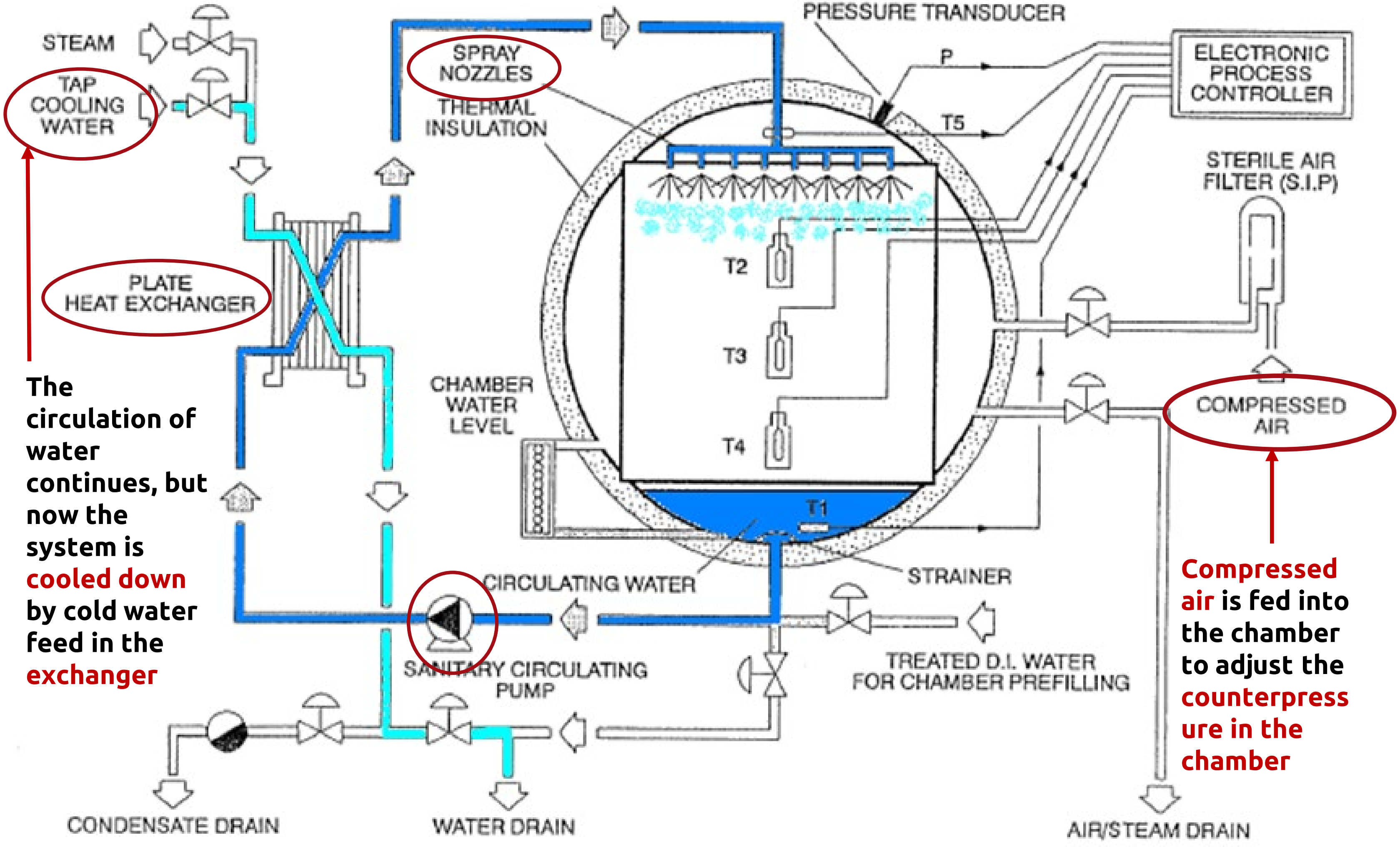
Heating and Sterilization



Steam is fed into **heat exchanger** and the whole system (including the load) is heated and kept hot

Compressed air is fed into the chamber to adjust the **counterpressure in the chamber**

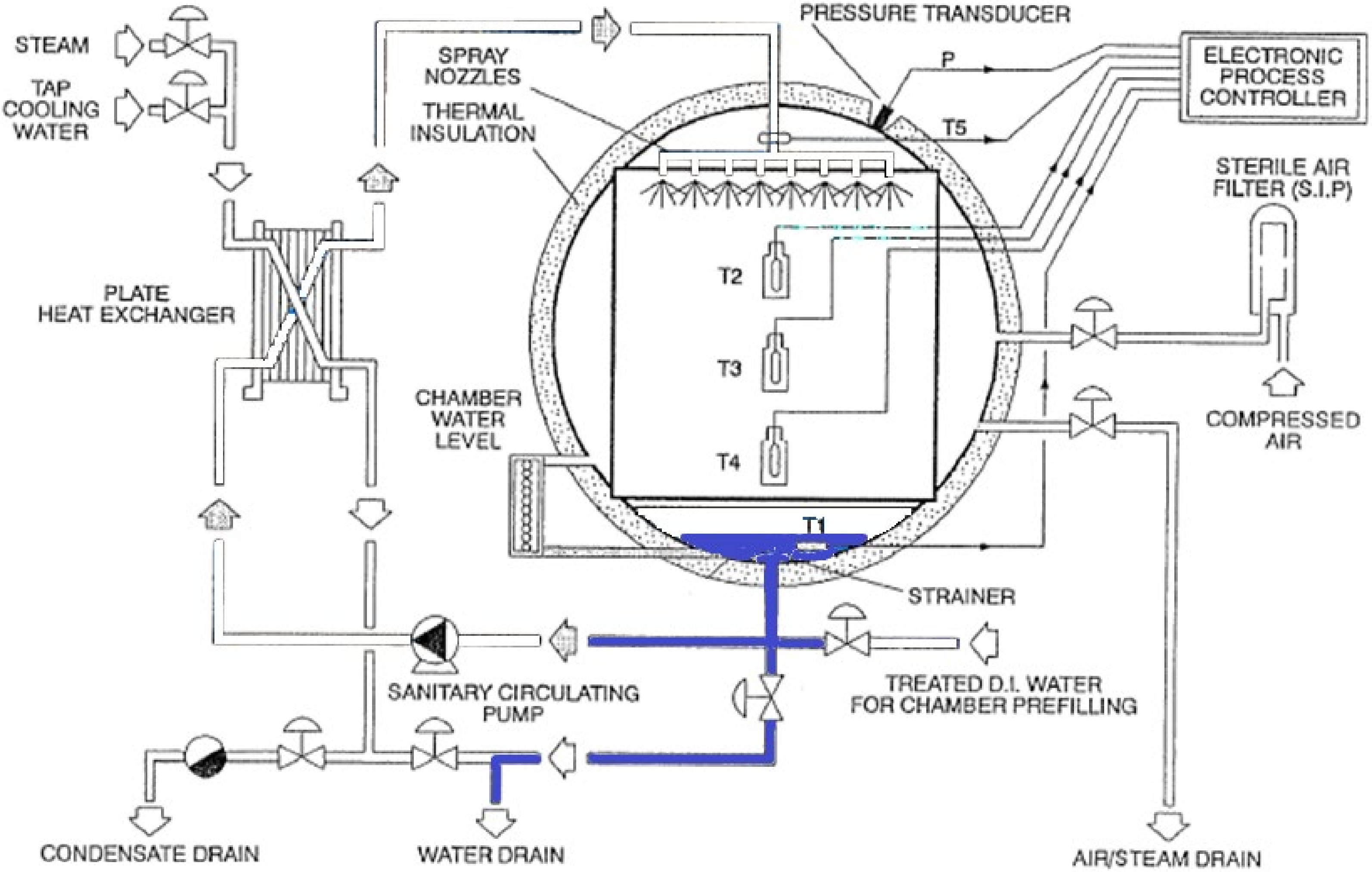
Cooling



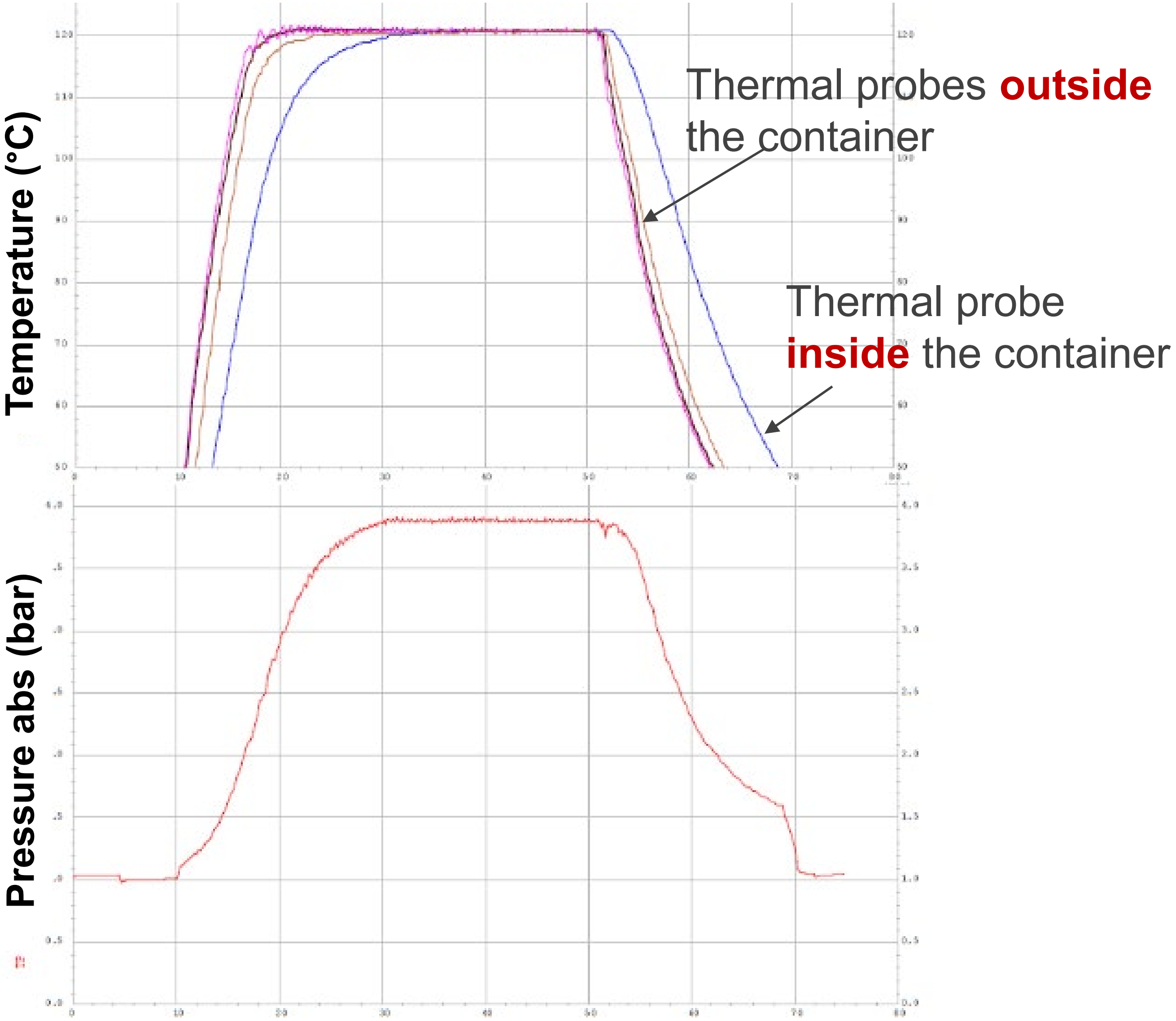
The circulation of water continues, but now the system is cooled down by cold water feed in the exchanger

Compressed air is fed into the chamber to adjust the counterpressure in the chamber

Chamber drain



Example of sterilization cycle

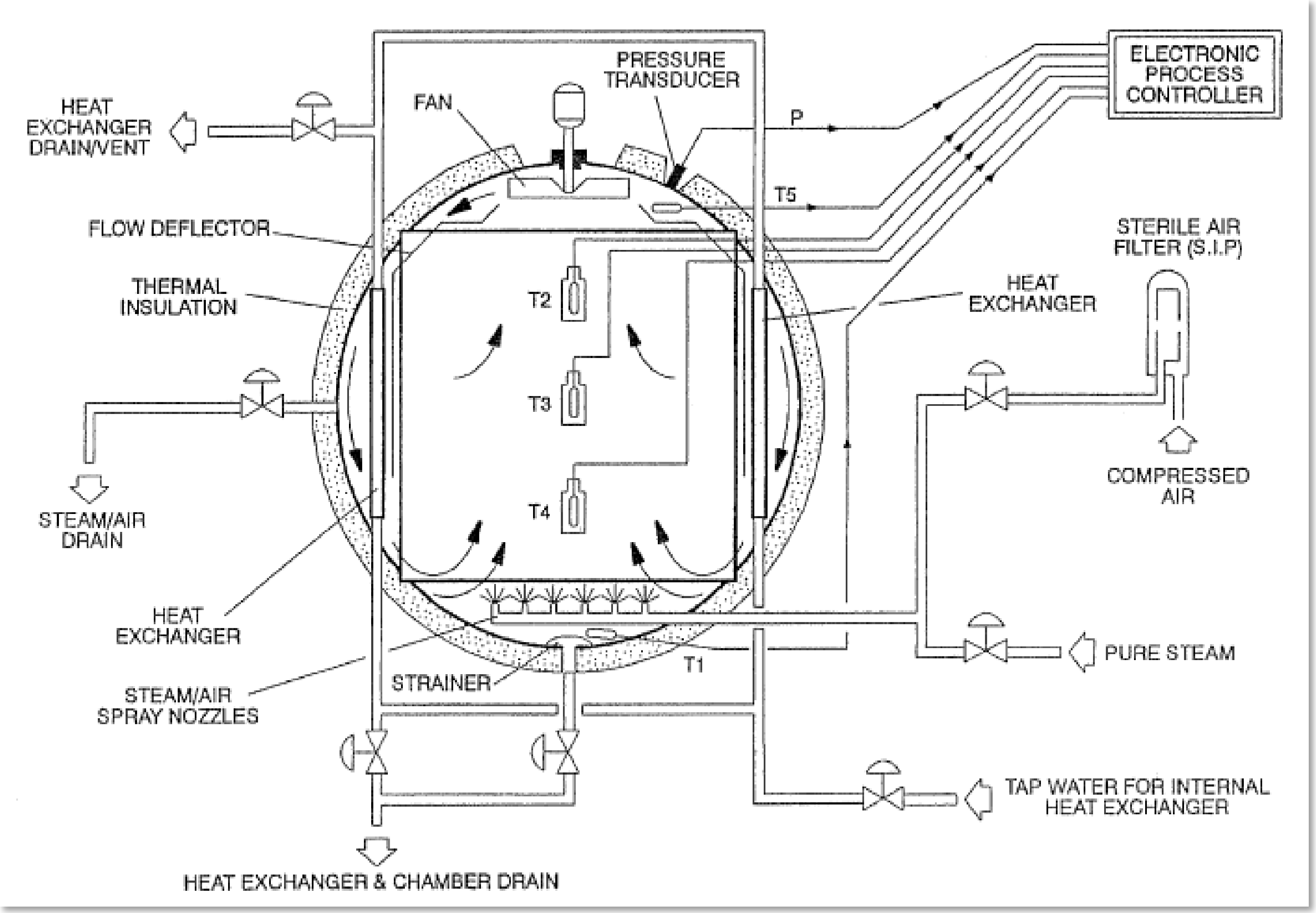


Thermal probes **outside** the container

Thermal probe **inside** the container

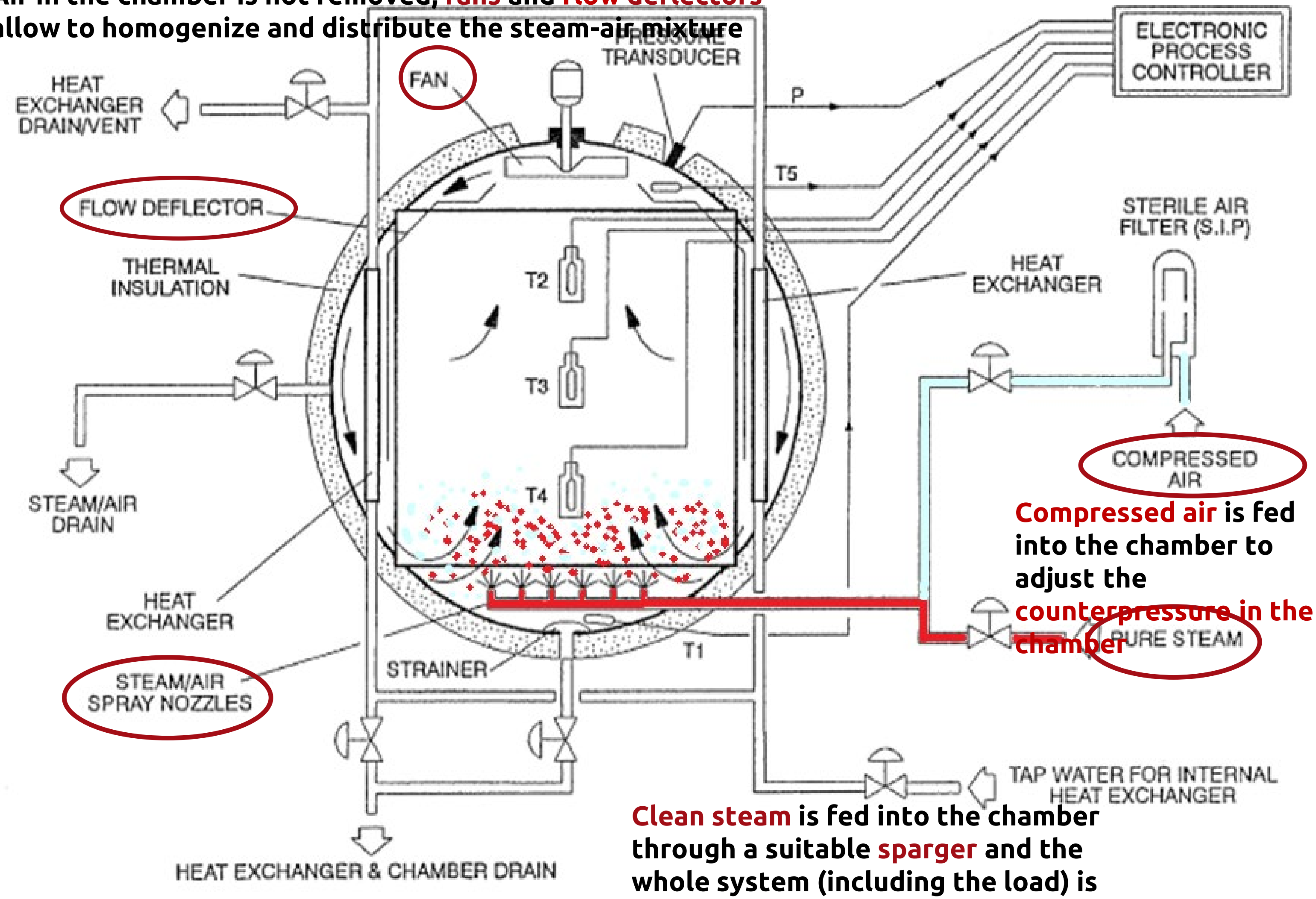
Steam-air mixture autoclave

Steam-air mixture autoclave – schematic



Heating and Sterilization

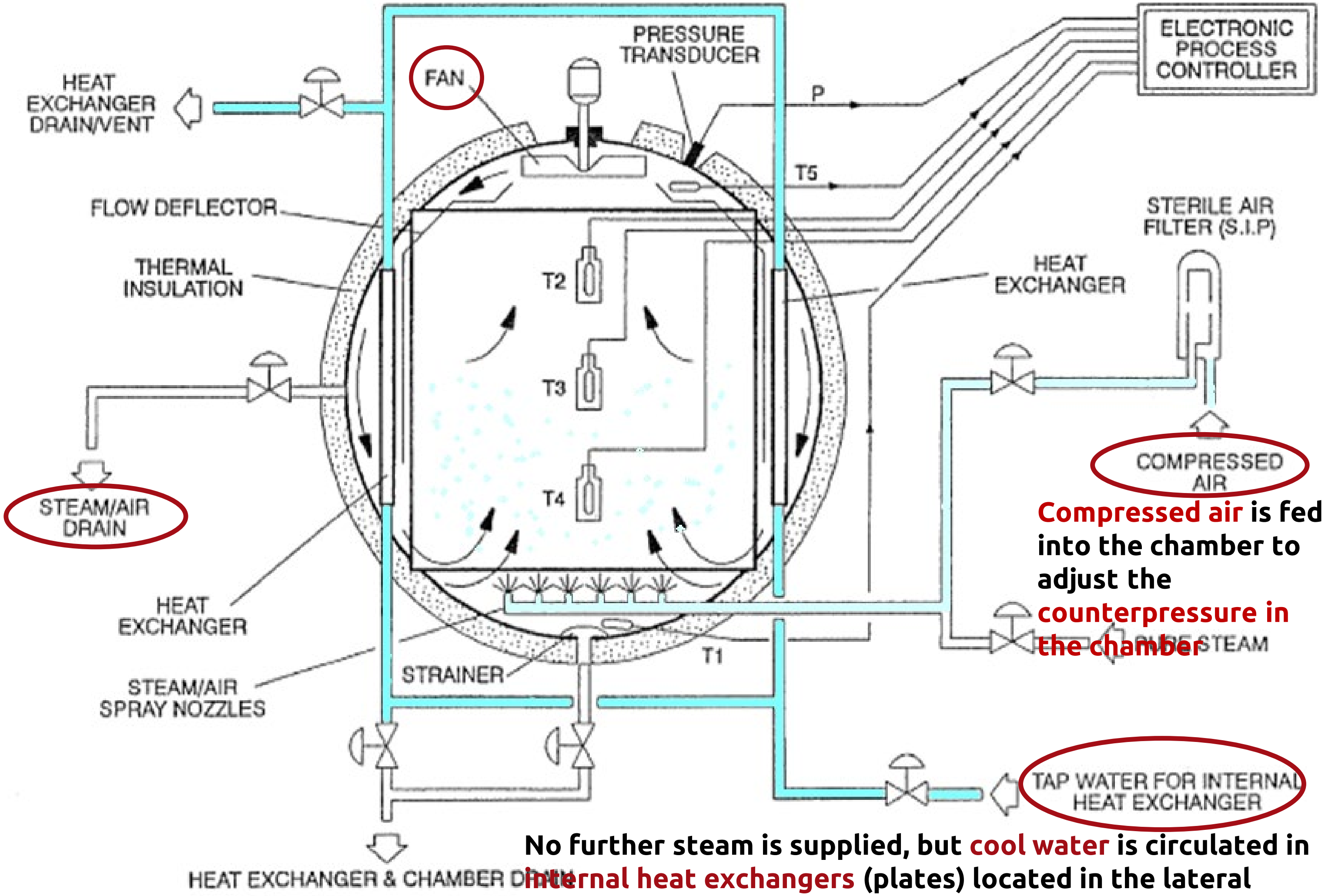
Air in the chamber is not removed; fans and flow deflectors allow to homogenize and distribute the steam-air mixture



Compressed air is fed into the chamber to adjust the counterpressure in the chamber

Clean steam is fed into the chamber through a suitable sparger and the whole system (including the load) is heated and kept hot

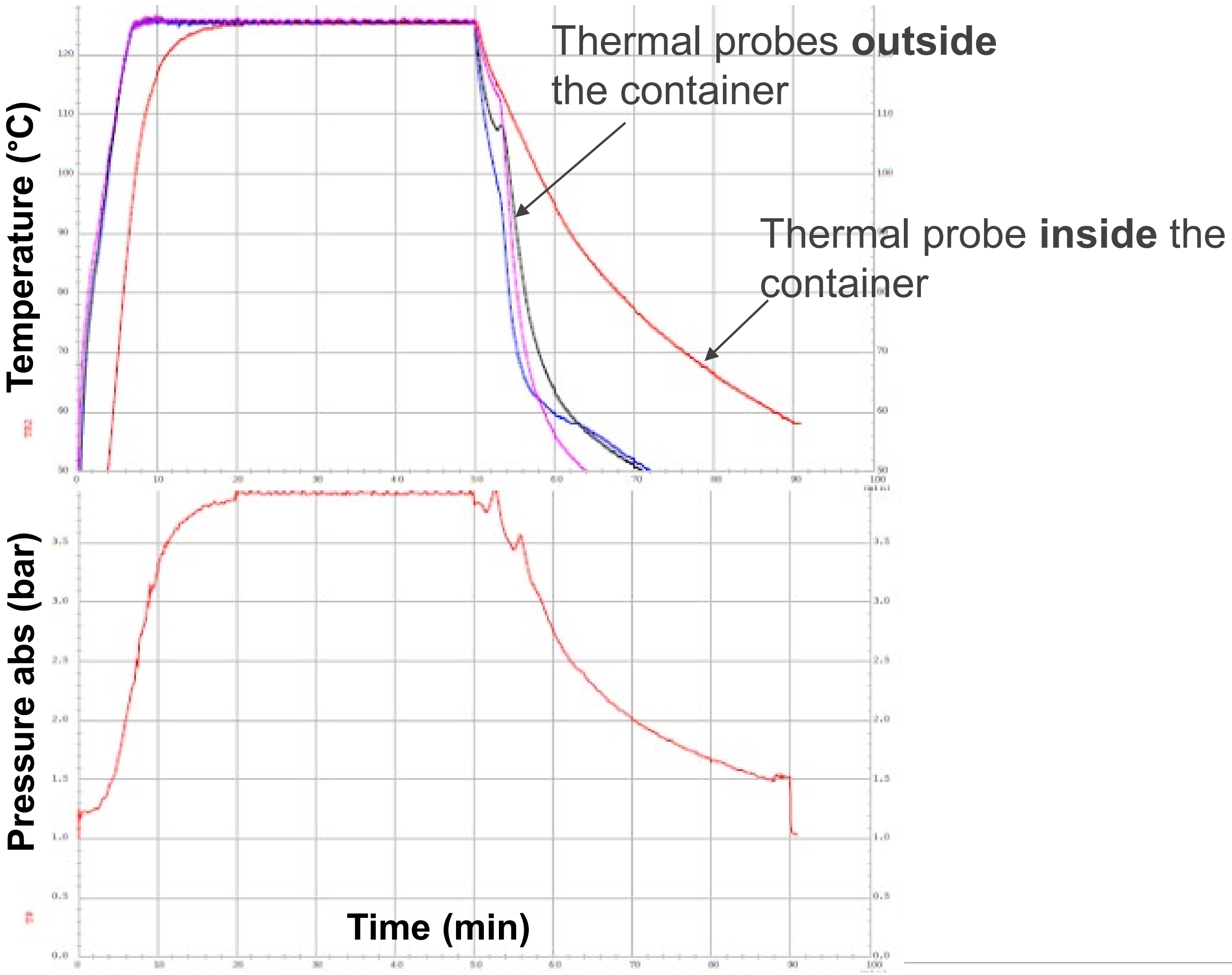
Cooling



Compressed air is fed into the chamber to adjust the **counterpressure in the chamber**

No further steam is supplied, but cool water is circulated in internal heat exchangers (plates) located in the lateral sectors of the chamber

Example of sterilization cycle



Thermal probes **outside** the container

Thermal probe **inside** the container

Post sterilization phases for liquids in sealed containers

Dont use a fast cooling phase immediately after sterilization of sealed containers

Why?

Fast cooling will cause a sudden vapour condensation with a dangerous pressure drop inside the autoclave

However…

The pressure inside the containers remains high because the temperature of the solution inside the containers falls slowly

Unloading hot solutions in general (in sealed and open containers)

Pressure shock **Thermal shock**

↘ ↙

Risks of burns caused by:

boiling or **overflow** of the **solution** (open container)

explosion («cascade effect») (sealed container)

↓

Temperature of the solution must be below 70°C (ideally <65°C) before the container is removed from the autoclave

Summary (Cooling for liquids) in sealed containers

Cooling under pressure is always used but…

Superheated Water Autoclave:

DIRECT cooling by water spray

- Product is unloaded wet
- The cooling phase is faster (heat exchange occurs through a liquid: more efficient)

Steam-Air Mixture Autoclave:

INDIRECT cooling by forced air circulation (fan) + cold water circulation in the plates and jacket (if present)

- Product can be unloaded dry
- The cooling phase is longer (heat exchange occurs through a gas: less efficient)

A comparison…

Superheated Water Process

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

Steam-Air Mixture Process

- – Controlled modulated heating but not possible modulated cooling
- – Longer process duration (mainly because of indirect cooling)
- – Consumption of clean steam
- + Product could be easily unloaded dry
- + No PW/UPW/WFI water consumption
- + Blushing phenomenon very rare

Case Studies

Liquids in plastic sealed containers

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 :

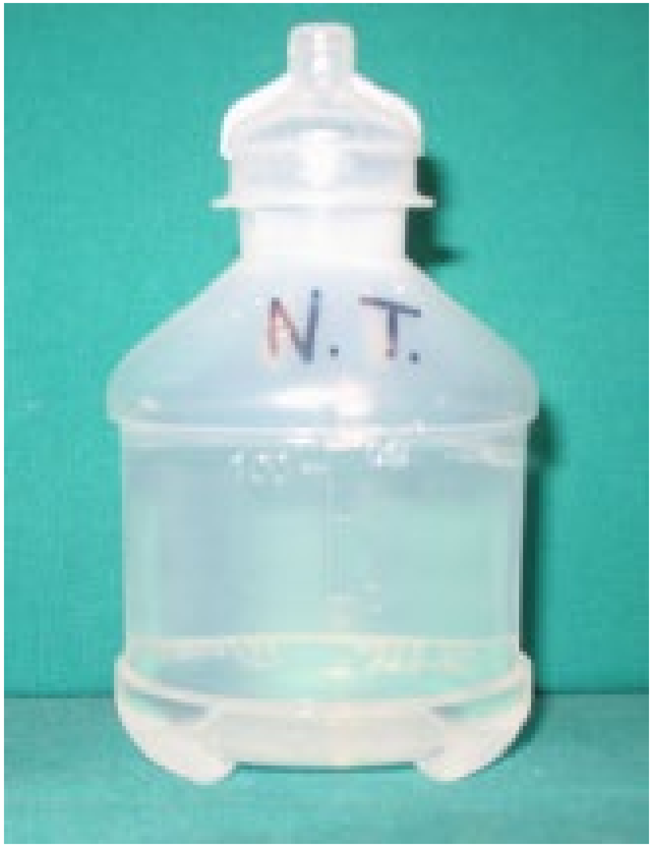
100 mL plastic bottles

Autoclave type:

Superheated Water

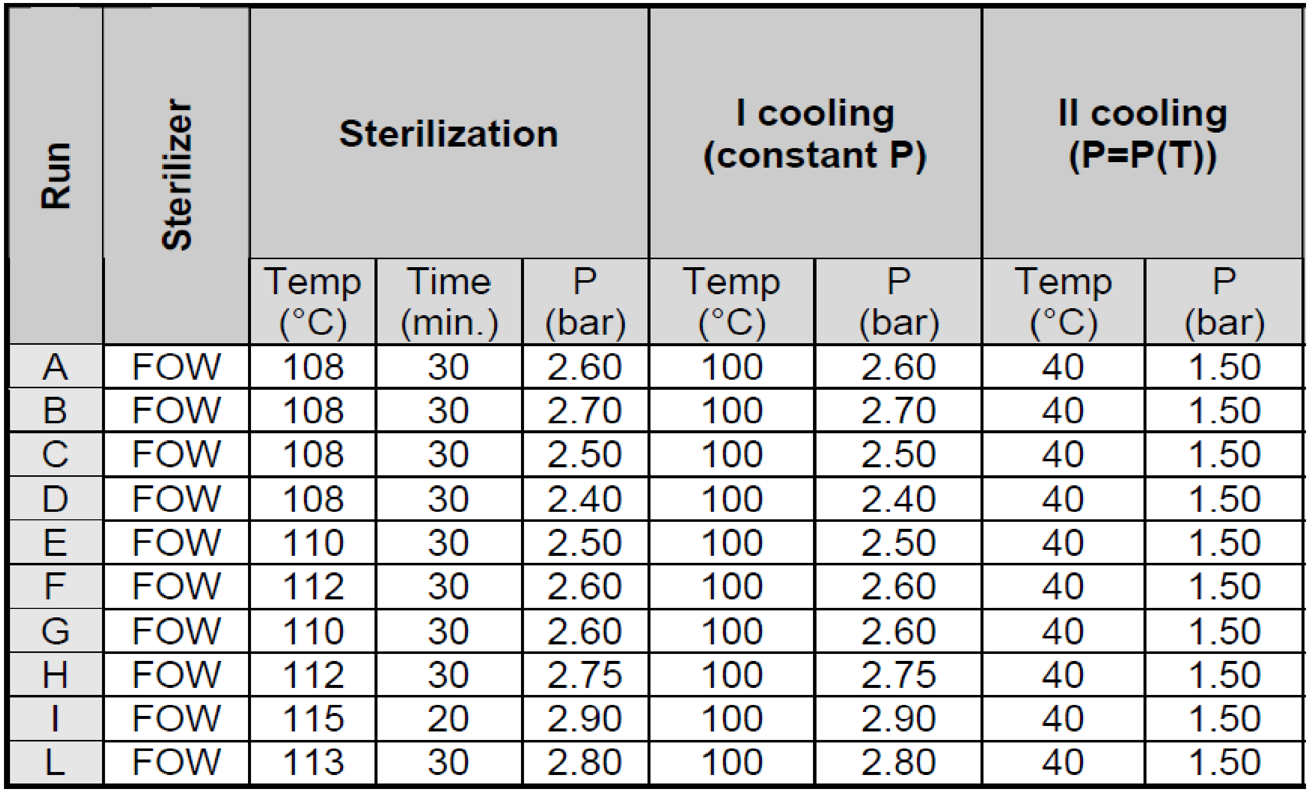
Challenge:

container deformation



Liquids in plastic sealed containers

Treatments at different temperatures (108-115°C) and counterpressures (2.4 -2.9 bar abs)



Run	Sterilizer	Sterilization			I cooling (constant P)		II cooling (P=P(T))	
		Temp (°C)	Time (min.)	P (bar)	Temp (°C)	P (bar)	Temp (°C)	P (bar)
A	FOW	108	30	2.60	100	2.60	40	1.50
B	FOW	108	30	2.70	100	2.70	40	1.50
C	FOW	108	30	2.50	100	2.50	40	1.50
D	FOW	108	30	2.40	100	2.40	40	1.50
E	FOW	110	30	2.50	100	2.50	40	1.50
F	FOW	112	30	2.60	100	2.60	40	1.50
G	FOW	110	30	2.60	100	2.60	40	1.50
H	FOW	112	30	2.75	100	2.75	40	1.50
I	FOW	115	20	2.90	100	2.90	40	1.50
L	FOW	113	30	2.80	100	2.80	40	1.50

Liquids in plastic sealed containers

Standard cycle with ‘Superheated Water’ autoclave

No.	Phase
1	PREPARE AUTOCLAVE
2	CHAMBER H20 FILL
3	H20 CIRCULATION
4	STABILIZATION
5	HEATING
6	STERILIZATION
7	COOLING
8	COOLING EXTENSION
9	COOLING
10	COOLING EXTENSION
11	CHAMBER DRAIN
12	ATMOSPHERIC BALANACE AND EXCHANGER DRAIN
13	CYCLE END

Two consecutive stages of cooling in order to mantain a constant pressure when the temperatures are higher and then a faster one when the temperatures are lower

Liquids in plastic sealed containers

- ✓ Load :
  500 mL plastic bags
- ✓ Autoclave type:
  Steam-Air Mixture
- ✓ Challenge:
  bag integrity

Liquids in plastic sealed containers

Standard cycle with ‘Steam-Air Mixture’ autoclave

No.	Phase
1	PREPARE AUTOCLAVE
2	HEATING WITH P=P(T)
3	STERILIZATION WITH P=P(T)
4	PRESSURIZE CHAMBER BY AIR
5	CONTROLLED RATE COOLING
6	COOLING EXTENSION
7	WATER DRAIN
8	PRESSURIZE CHAMBER BY AIR
9	CONTROLLED RATE COOLING
10	COOLING EXTENSION
11	WATER DRAIN
12	ATMOSPHERIC BALANACE AND EXCHANGER DRAIN
13	CYCLE END

Two consecutive stages of cooling in order to mantain a constant pressure when the temperatures are higher and then a faster one when the temperatures

Liquids in plastic sealed containers

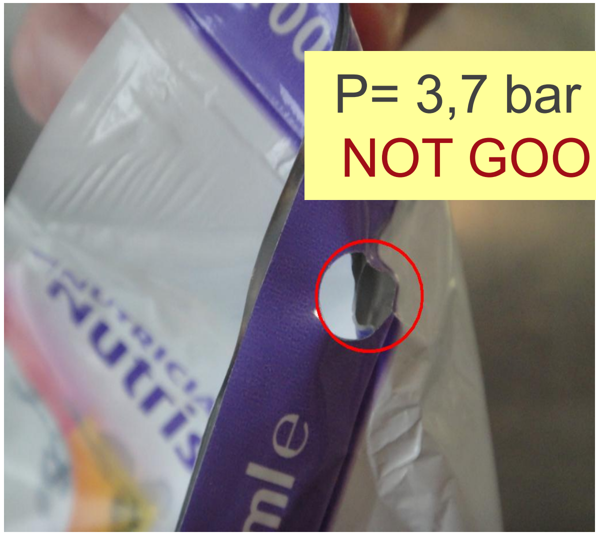
Sterilization phase:

T= 126°C

P= 3.9 bar abs (k=1,2)

The bags break if the applied conterpressure is lower

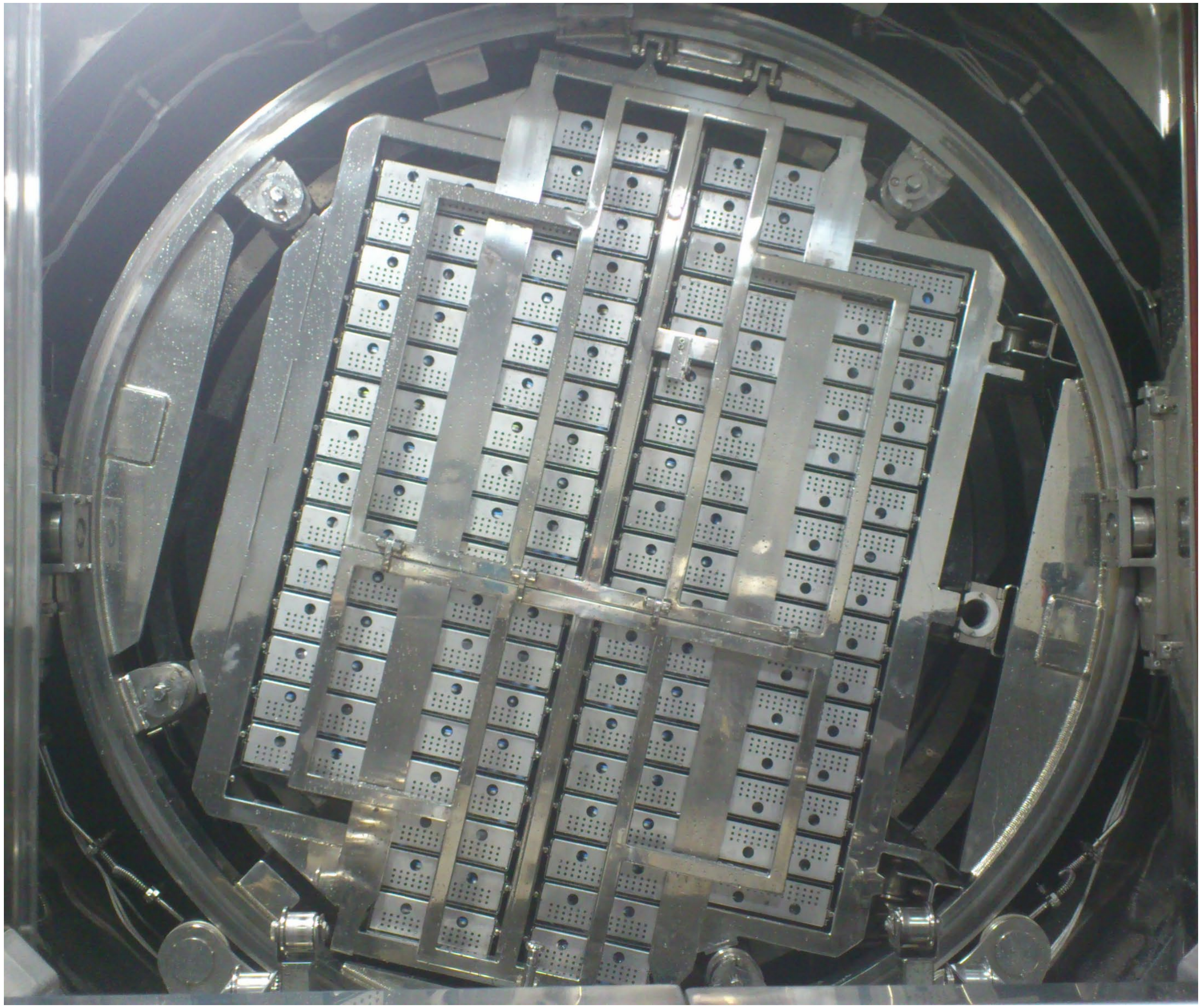
Fast indirect cooling with water circulation in jacket and plates + air circulation with fan



P= 3,7 bar abs

NOT GOOD!!!

Rotating basket for liquids: why?



Rotating basket for liquids: why?

Emulsions, Suspensions

Dense or non homogeneous mixtures

Heat sensitive products (sometimes)

Rotating basket: suspension in glass vials

The load can be rotated during the sterilization treatment in order to:

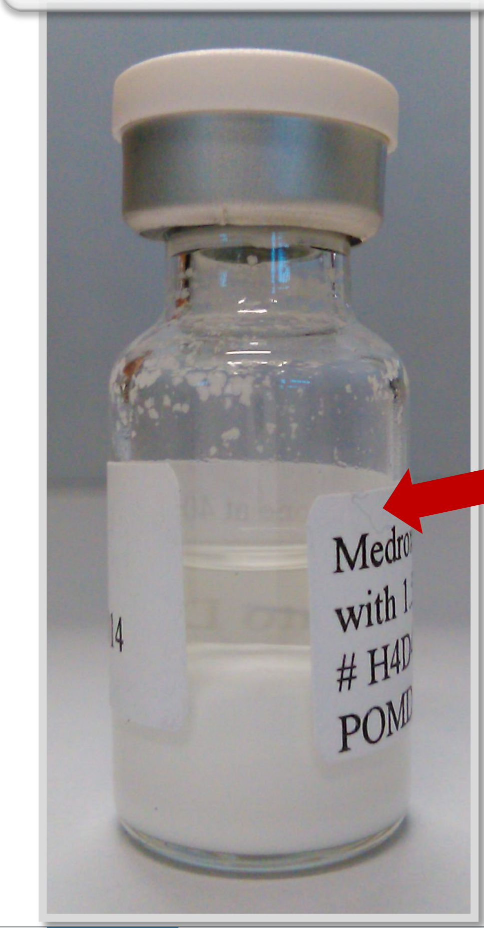
Increase the speed of heating in case of dense or non homogeneous mixtures

Maintain the stability / homogeneity of emulsions and / or suspensions

Improve the heat exchange during the heating/cooling phases (ex. to treat heat-sensitive products at a high temperature for a short time)

Rotating basket: suspension in glass vials

SAMPLE A



✓ Load :
3 ml glass vials sealed
with rubbers stoppers
and flip-off caps.
1 ml suspension: same
antitumor drug but two
✓ types of formulations

✓ Autoclave type:
Superheated Water

✓ Challenge:
Sterilization and stability
of the suspension

SAMPLE B

Rotating basket: suspension in glass vials

Load treatment strategy

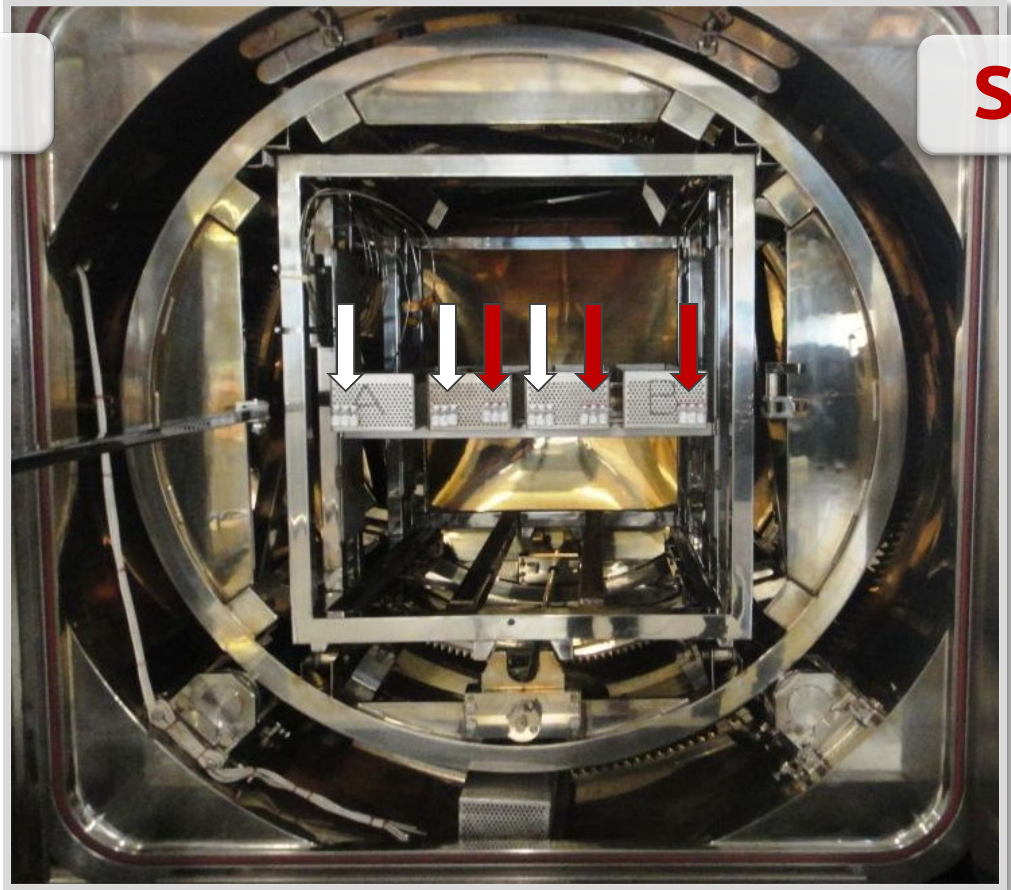
- Autoclave type: superheated water sterilizer
- Sterilization phase at 121°C:

  controlled by $F_{0\ \mathrm{bio}}$ target (= 8 min)
- Load configuration: the vials were located in the middle and at the lateral side of the rotating trolley
- Rotation rate: 1,5-2-6-8 rpm

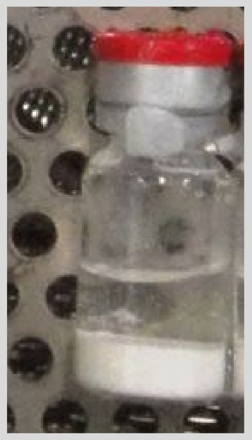
Rotating basket: suspension in glass vials

Load treatment strategy

SAMPLE A



SAMPLE B



Rotating basket: suspension in glass vials

Results

- The location of the samples did not influence the results
- Rotation speed of 1,5 rpm was enough to mix the two phases of sample A, whereas, for sample B, the two phases were never completely mixed together

Rotating basket: suspension in glass vials

After treatment (rotation speed: 1,5 rpm)

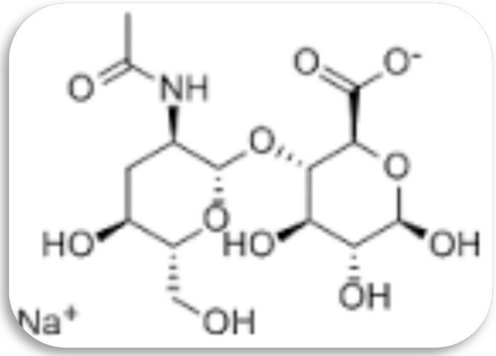
SAMPLE A
The two phases
are mixed

SAMPLE B
The two phases
are separated

Liquids in plastic sealed containers

PFS with sodium hyaluronate

Glass pre-filled syringes containing sodium hyaluronate gel



Liquids in plastic sealed containers

PFS with sodium hyaluronate

Heat-sensitive product

Its molecule might break, affecting therapeutic efficiency or creating toxic compounds

Related effects:

- Viscosity is reduced
- Colour changes

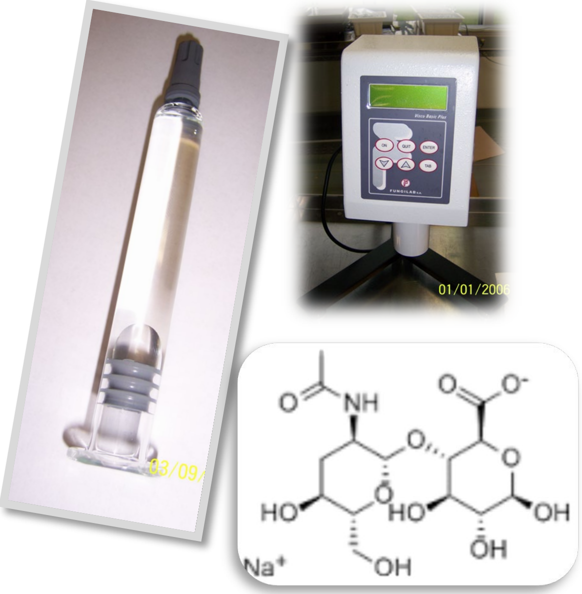
Liquids in plastic sealed containers

PFS with sodium hyaluronate

Load treatment objectives and challenges

- Autoclave type: steam-air mixture (FOA)
- Sterilization phase:

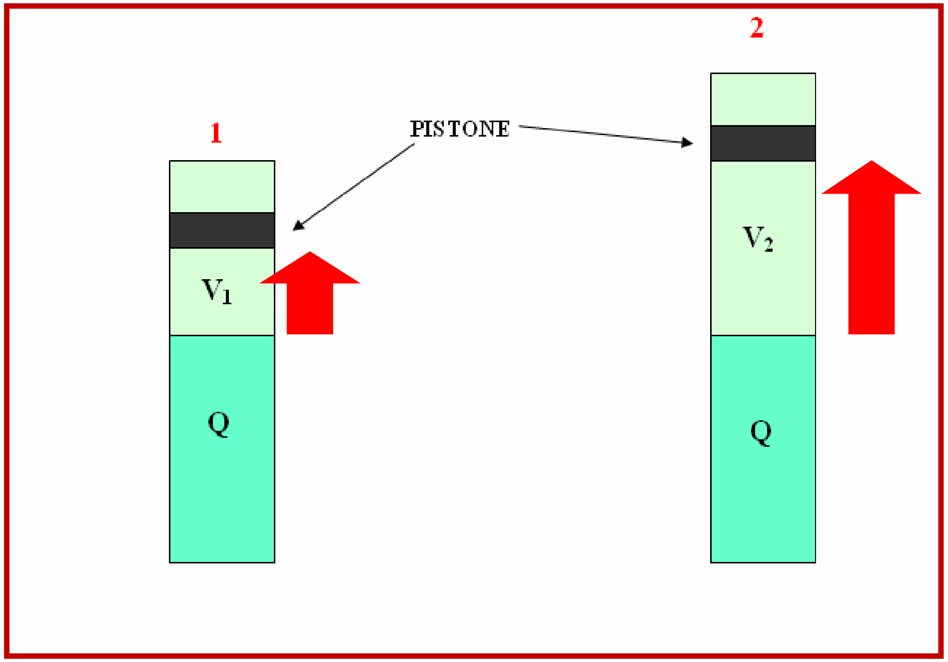
  controlled by F_0 target = 12 min
- Minimize product viscosity reduction



PFS with sodium hyaluronate

Container sealed with a plunger

⇒ variable volume container



Q= amount of liquid

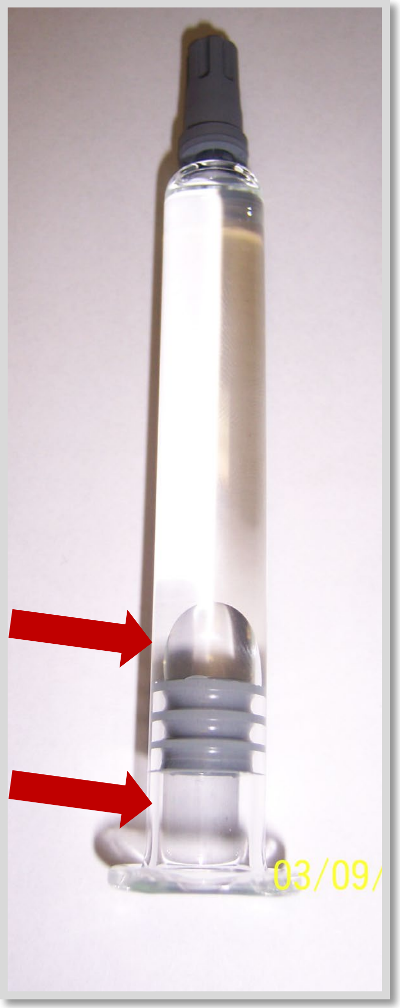
V_1= head space volume for container 1

V_2= head space volume for container 2

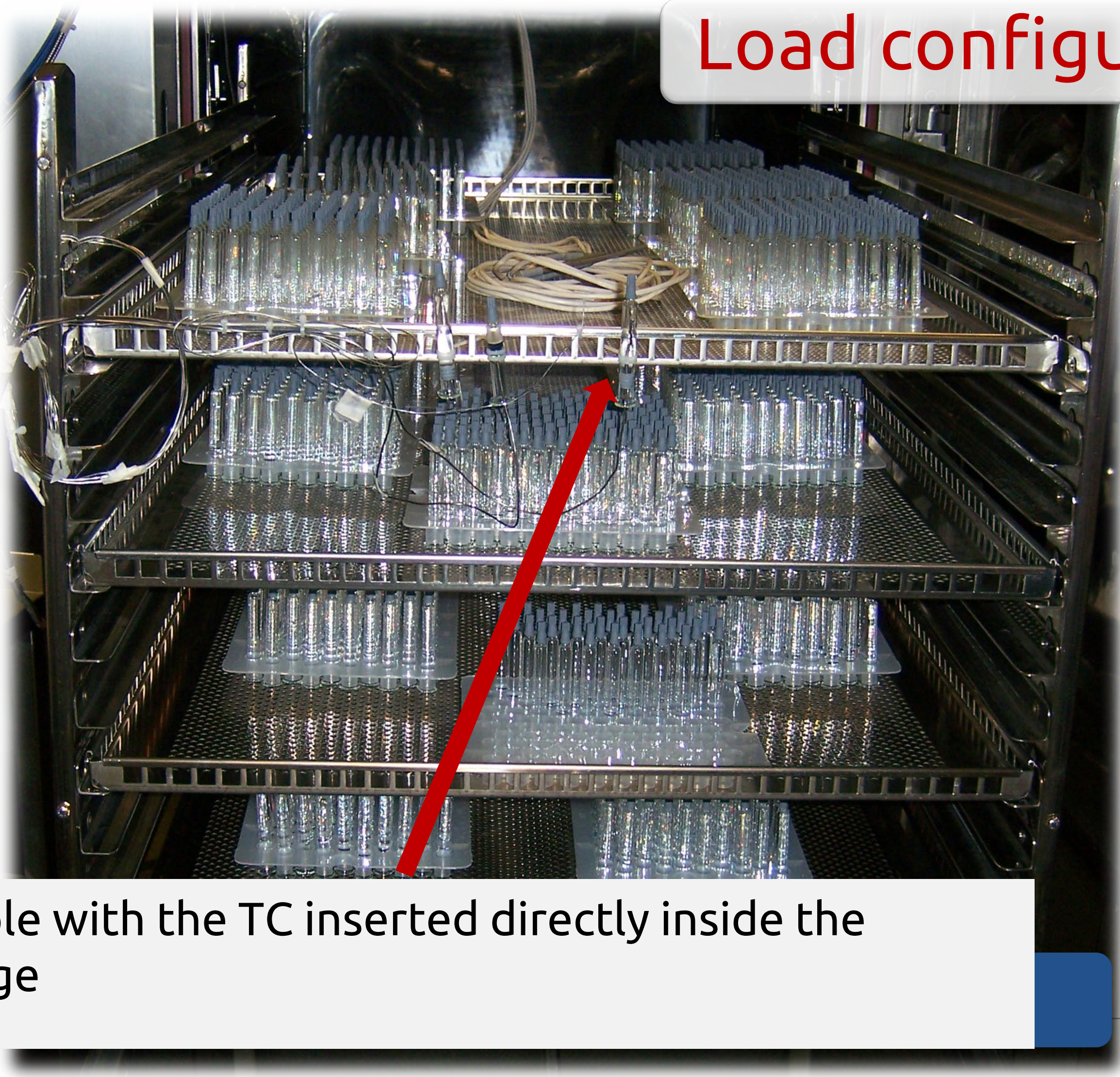
PRESSURE on PLUNGER 1 > PRESSURE on PLUNGER 2

PFS with sodium hyaluronate

The head space and the space behind the plunger is small



PFS with sodium hyaluronate



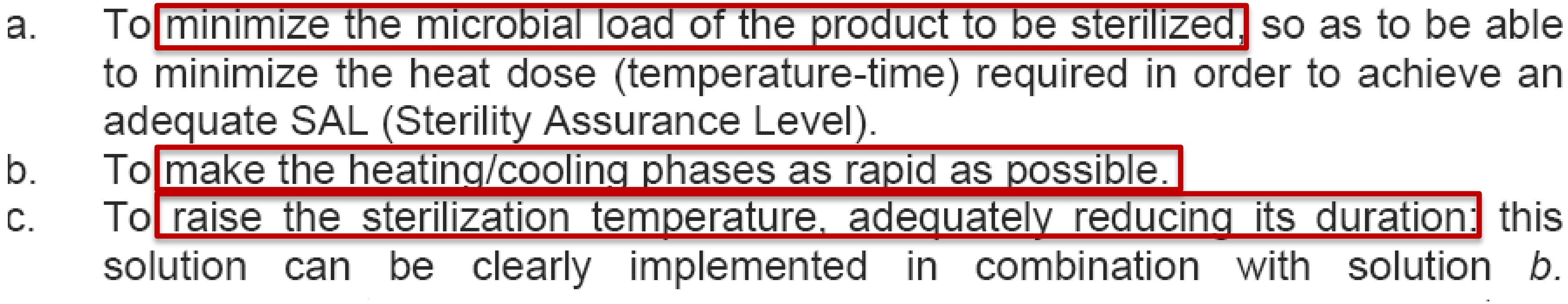
Load configuration

Sample with the TC inserted directly inside the syringe

PFS with sodium hyaluronate

Load treatment strategy: viscosity reduction

- Possible strategies to treat heat-sensitive loads



a. To minimize the microbial load of the product to be sterilized, so as to be able to minimize the heat dose (temperature-time) required in order to achieve an adequate SAL (Sterility Assurance Level).

b. To make the heating/cooling phases as rapid as possible.

c. To raise the sterilization temperature, adequately reducing its duration: this solution can be clearly implemented in combination with solution *b.*

PFS with sodium hyaluronate

Load treatment strategy: viscosity reduction

Load treatment strategy: viscosity reduction

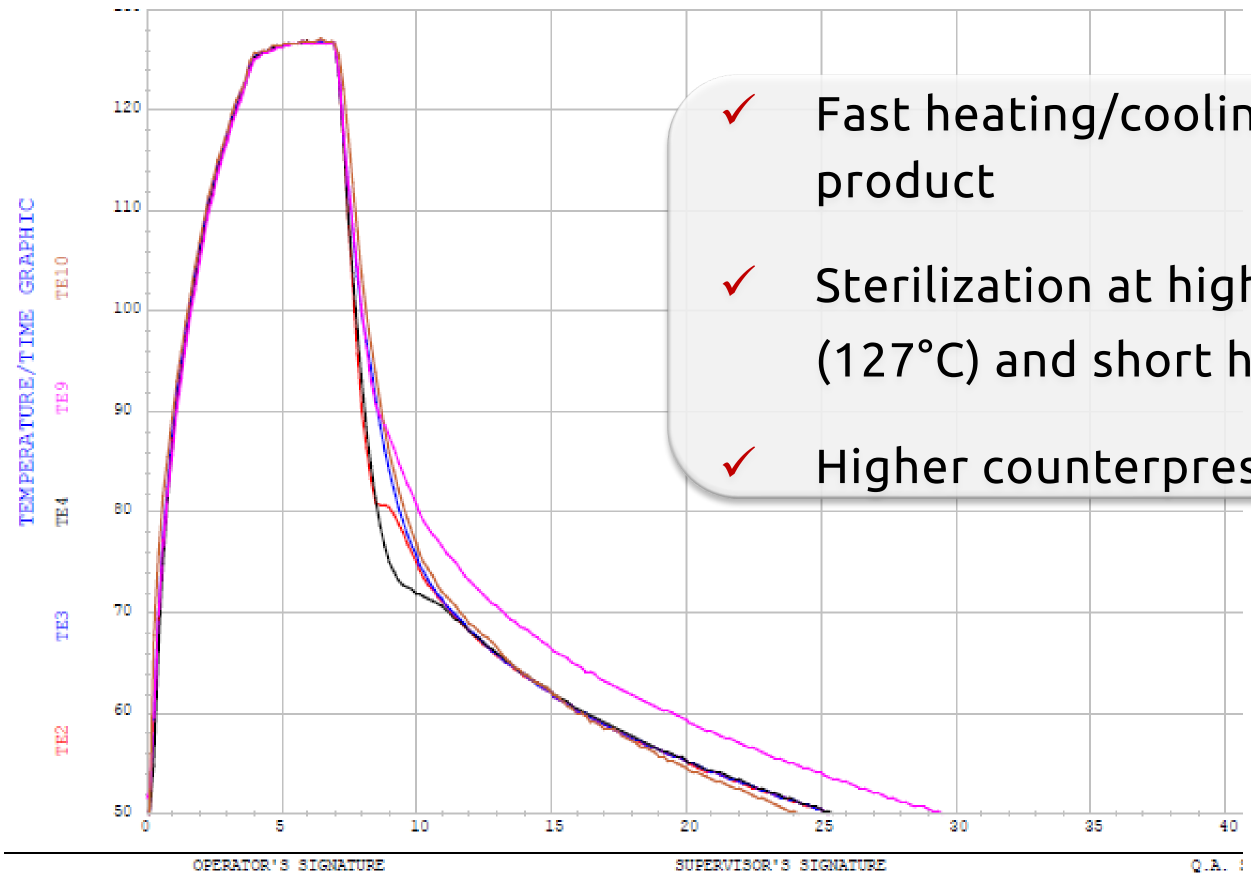
Higher sterilization temperature for a shorter time

↓ Why ?

The rate of inactivation of microorganism increases with the increment of temperature much faster that the rate of thermal degradation

PFS with sodium hyaluronate

Developed cycle to minimize viscosity reduction and avoid plunger expulsion



- ✓ Fast heating/cooling of the product
- ✓ Sterilization at high temperature (127°C) and short holding time
- ✓ Higher counterpressure

PFS with sodium hyaluronate

Results

- The plunger was not forced out
- The viscosity reduction was lower for the samples treated at 127° C

01/01/2006

Sterilization temperature (F_0 target = 12’)	Viscosity reduction
Not treated sample	0%
121°C	64%
127°C	42%

Control of the process: Saturated Steam vs Counterpressure

Saturated Steam

- The **control** of the process is based on the **pressure**, that is then converted into a temperature value;
- **Chamber pressure depends** on the **temperature**

Counterpressure

- The **control** of the process is based on the **temperature** (thermal probe)
- **Chamber pressure** is **independent** from the **temperature** (in order to reach a pressure higher than the one of saturated steam at the same temperature, thus balancing the pressure inside the sealed container with the liquid)

Thank you.