Sterilization depyrogenation

Institute of Pharmaceutical Technology and Biopharmacy

Sterilization

Sterilization is an **operation** that can **remove/destroy** the micro-organisms **from/in** the product.

Aim: sterility of the preparation.

Sterility (sterile prod.): „is the absence of viable micro-organisms.”
(viable- and spore-forms)

Sterility control:

- proper, validied procedure (GMP)
- examination
- unopened product / container

Sterilization

Sterilization: the summation of operations and procedures, that can ensure sterility of the final-product.

„The chosen test **method depends on the properties of the material to be sterilized**.
Eventual heat-sensitivity must be taken into consideration, and sterility must be achieved without causing change in the therapeutic action.”

„**The sterility of a product cannot be guaranteed by testing; it has to be assured by the application of a suitably validated production process**.”

Sterilization

When a fully validated thermal sterilization method:

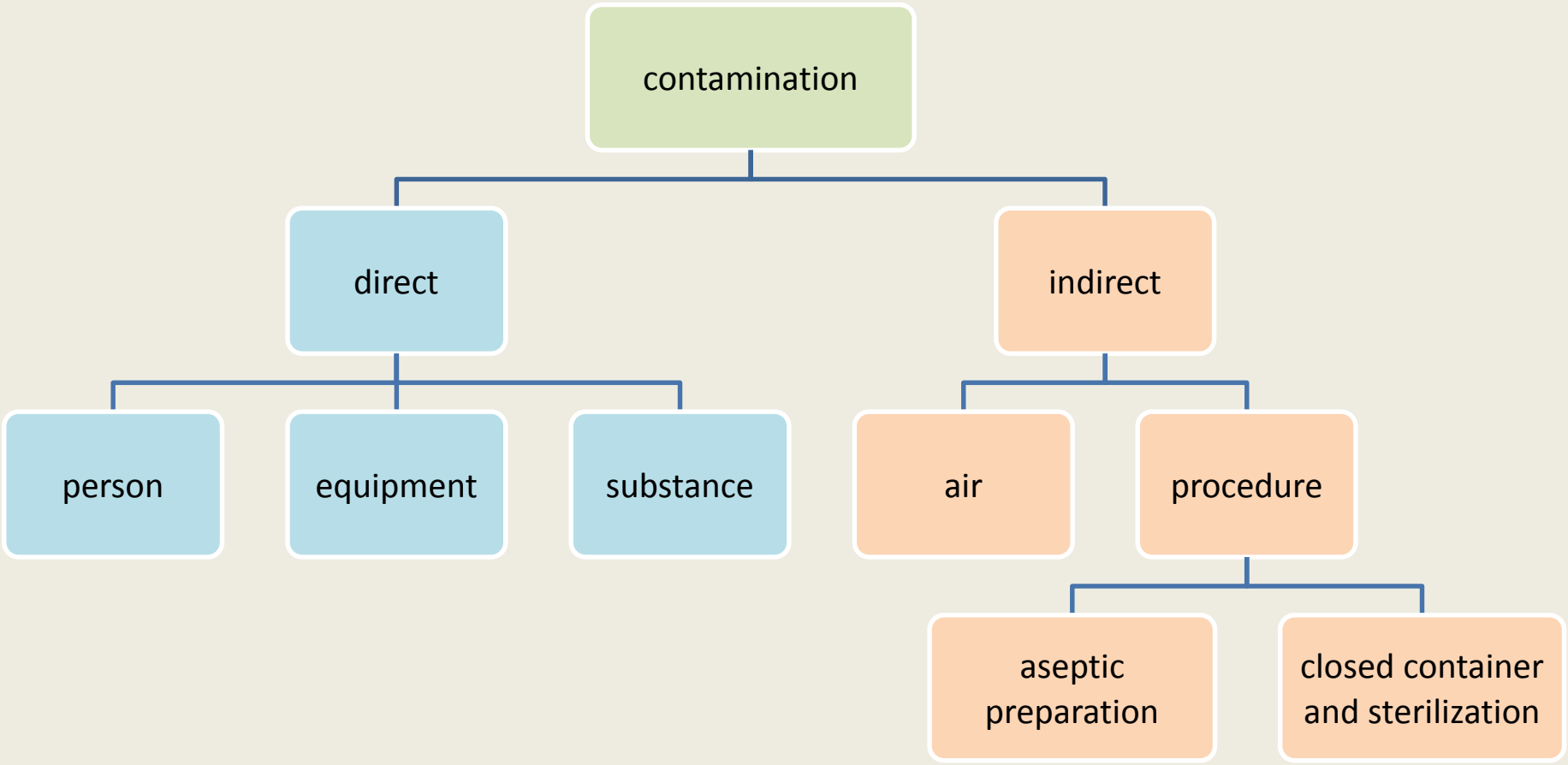
by steam, dry heat or ionising radiation is used, parametric release, that is a release of a batch of sterilized items based on process data rather than on the basis of submitting a sample of the items to sterility testing, may be carried out, subject to the approval of the competent authority.

OR:

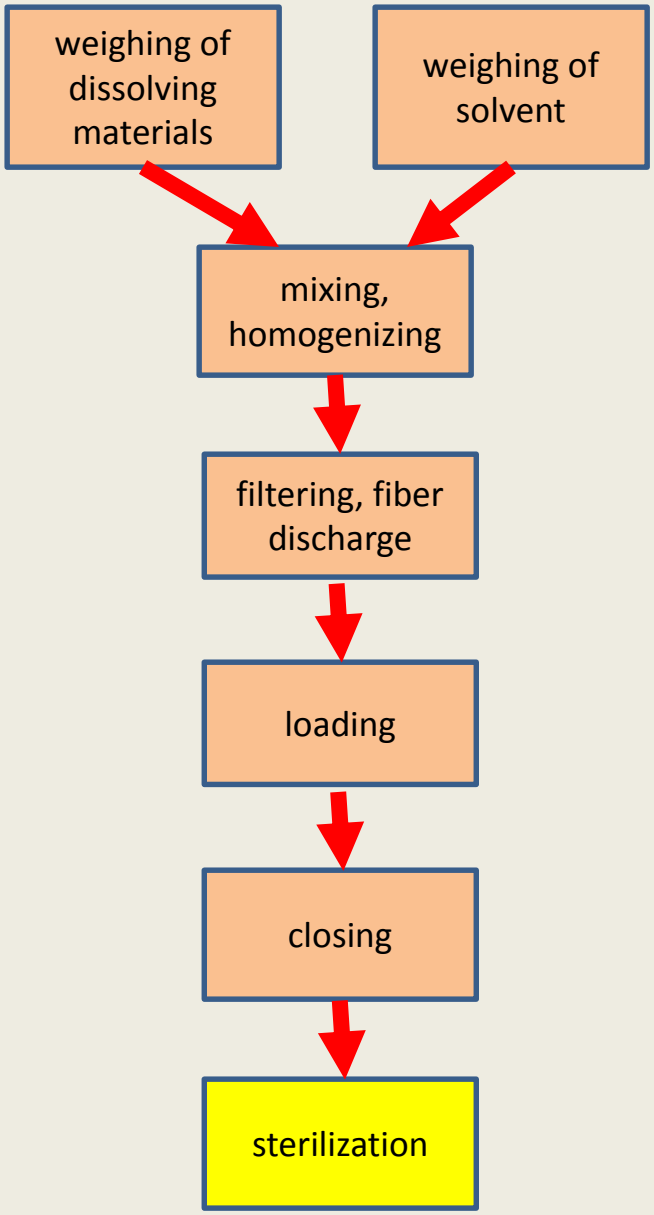
- „If thermal sterilisation is not possible, filtration trough a bacteria-retentative filter or aseptic processing is used; wherever possible, appropriate additional treatment of the product … in its final container is applied."

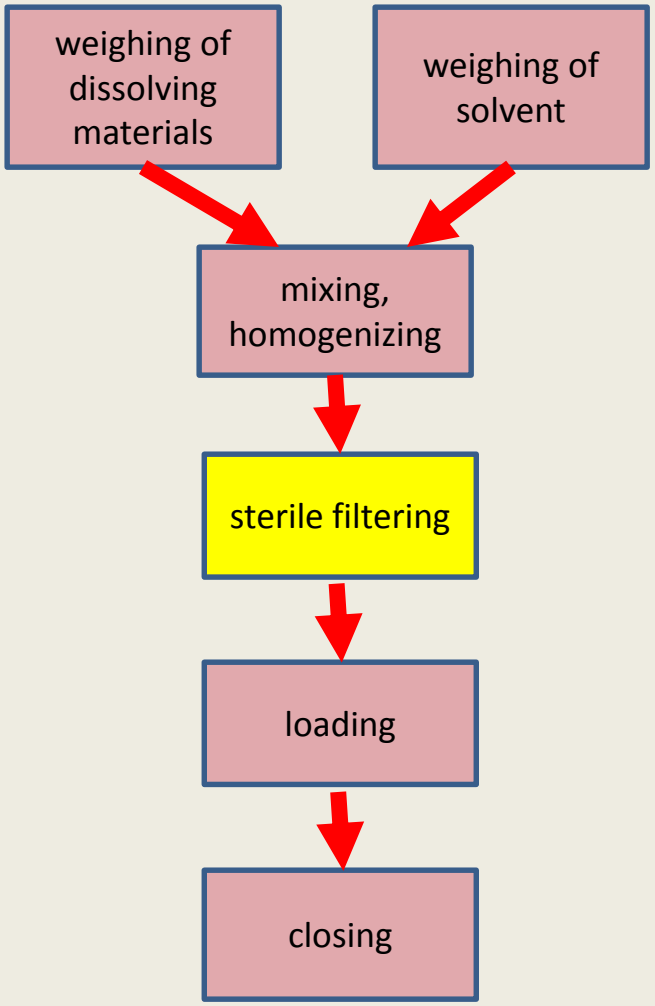
„In all cases, the container and closure are required to maintain the sterility of product throughout its self-time."

Contamination's options



Procedures


weighing of dissolving materials
weighing of solvent
mixing, homogenizing
filtering, fiber discharge
loading
closing
sterilization


weighing of dissolving materials
weighing of solvent
mixing, homogenizing
sterile filtering
loading
closing

Principles of sterilization

Micro-organisms

- Elimination: **filtration**
- Damage: physical methods: **heat, radiation**
  chemical methods: **oxydation**

Effectivness:

- Resistance of micro-organisms
- Number of bacteria
- Effectivness of the method

$$K = \frac{1}{t} \ln \frac{B}{b}$$

K = damage rate
t = sterilization time
B = numbers of bactaria at the beginning
b = numbers of bactaria at the t-time

Background of sterilization

Ph.Eur.6: „**SAL**" (**S**terility **A**ssurance **L**evel)

„The SAL of sterilizing process is the **degree of assurance** with which the process in question renders a population of items sterile."

„The SAL in a given process is expressed as the probability of non-sterile item in that population."

„An SAL of 10^{-6}, for example, denotes a probability of not more than one viable micro-organism in $1x10^{6}$ sterilized items of the final product."

The inactivation of micro-organosms follows an exponential statistic law.

$$K = \frac{1}{t} \ln \frac{B}{b}$$

K = damage rate
t = sterilization time
B = numbers of bactaria at the beginning
b = numbers of bactaria at the t-time

Definition of „Sterile”

- A sterilization process must deliver a **S**terility **A**ssurance **L**evel (SAL) of 1 in a million (10^{-6})
- It is not possible to measure 10^{-6}
- The required SAL can be achieved by **applying a process that will reduce the number of organisms to zero** and then apply a safety factor that will deliver an extra 6 log reduction

Background of sterilization

„The inactivation of micro-organisms by physical or chemical means follows an exponential law; thus there is always a finite statistical probability that a micro-organism may survive the sterilizing process.” .

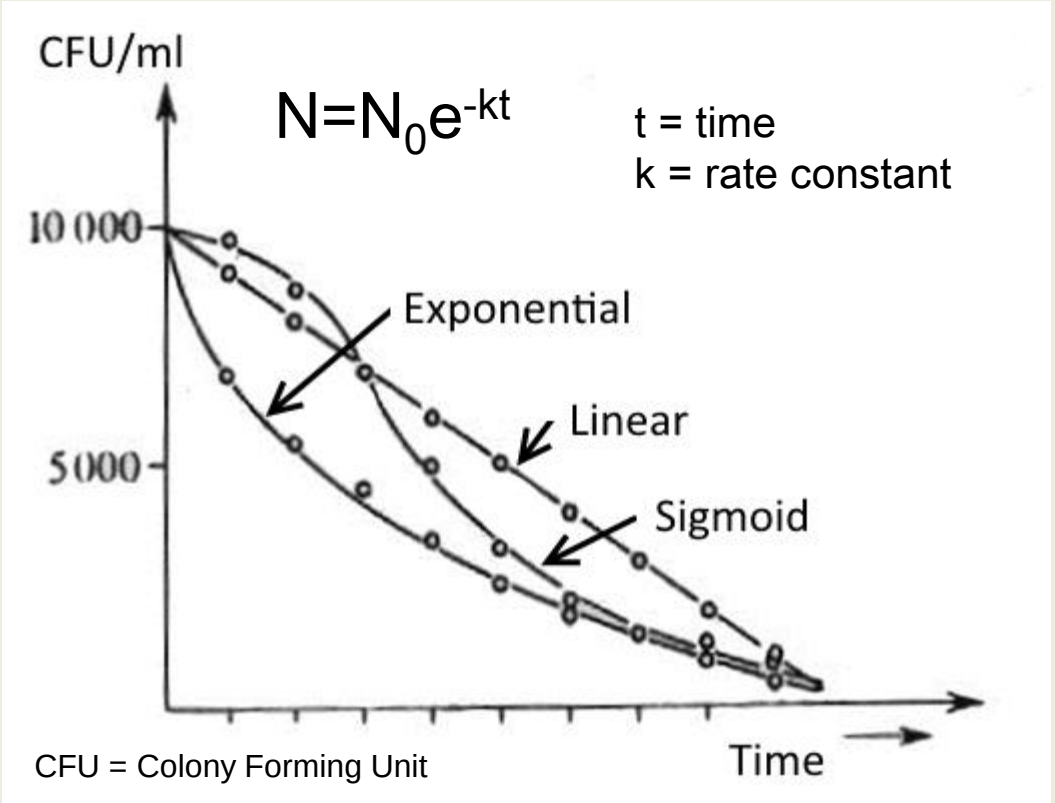
Higuchi-Buss ➔ $\ln t_d = \Delta H_s / R\,T + K$

ΔH_s = *necessary heat to sterilization in present one determined species*

K = species constant

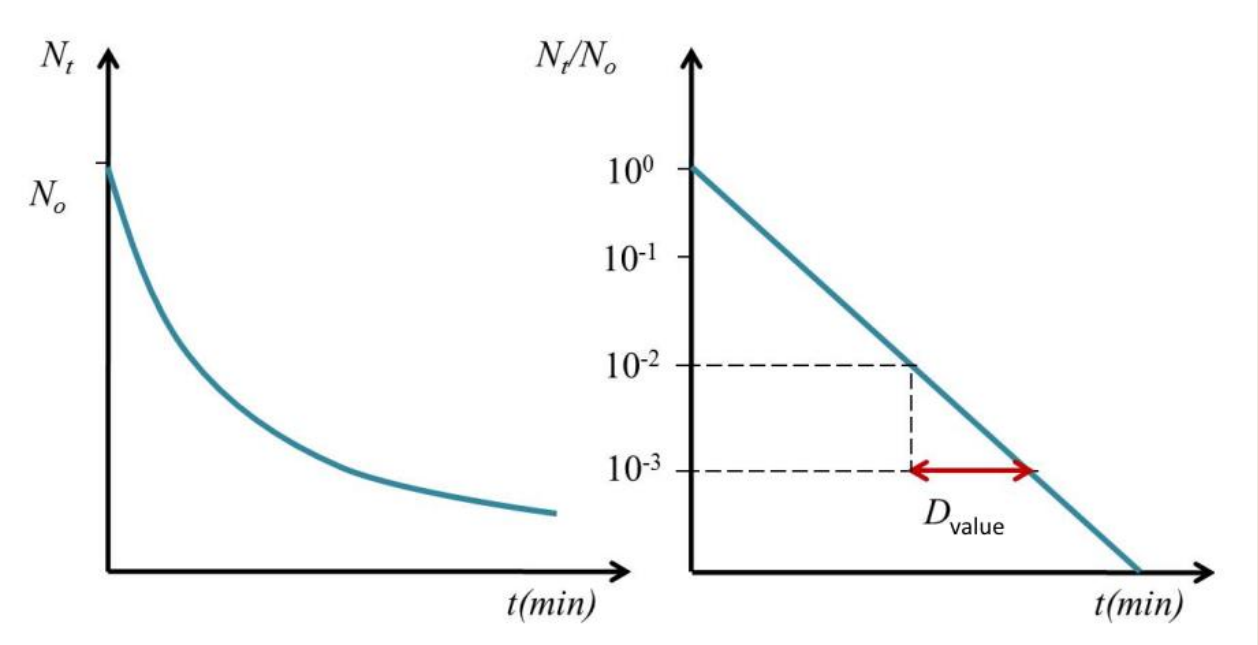
T_d = *sterilization time*

- numbers of micro-organisms (N),
- resistance,
- enviroment,
- Lower concentration of salts
- pH~7,
- Lower temperature



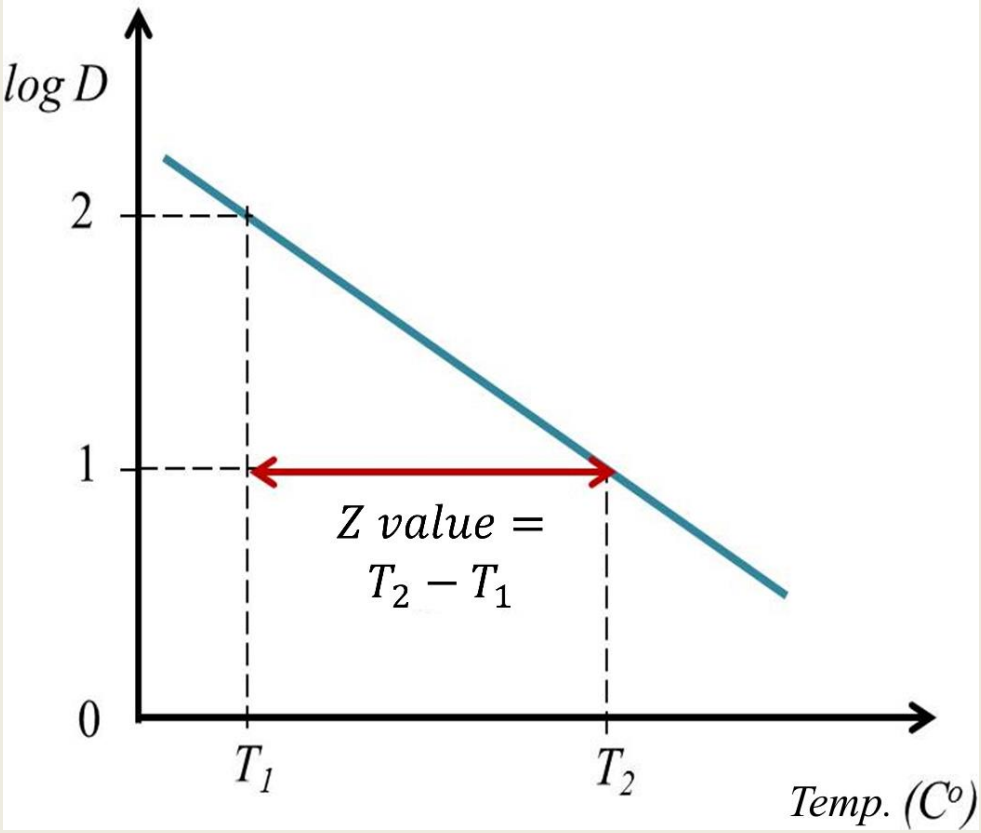
Definition of „Sterile”

Resistance of an organism is referred as its “**D-value**”



D-value - Time (or dose) **required to reduce the population of organisms by 1/10 of the initial number of microbes** (or 90%).

Definition of „Sterile”



The rate of heat death of microorganisms varies with temperature.
The Z value is the temperature change that changes the D value by an order of magnitude.

Methods of sterilization

I. Heat-sterilization

(flame sterilization (Ph. Hg. VI))

dry heat sterilization

steam sterilization

II. Radiation

IR, UV, ionisation

III. Filtration

adsorption and sieve effect

IV. Chemical routes

formaldehyde, β-propiolactone, ethylen-oxyde,

V. Plasm sterilization

hydrogen-peroxide plasm

Sterilization procedure

- Registration of the critical parameters (pressure and temperature) is important
- „The location within the sterilizing chamber that is least accessible to the sterilizing agent is determined for each loading configuration of each type and size of container or packege (*for example, the coolest location in an autoclave*).”
- It is advisable to take the sensors to the coolest parts of the chamber.
- Sensors can be filled into bottles, too.

Dry heat sterilization

(Ph.Hg.VII.)

Dry heat sterilization		
°C	Time	
	Ventillation (+/-)	
	+	-
200 ±5	10	35
180 ±5	25	60
160 ±5	45	120
140±5	-	180

Ph.Eur.6:

min. 160°C , min 2h

Proper reproductivity level
➔ SAL 10^{-6} or better

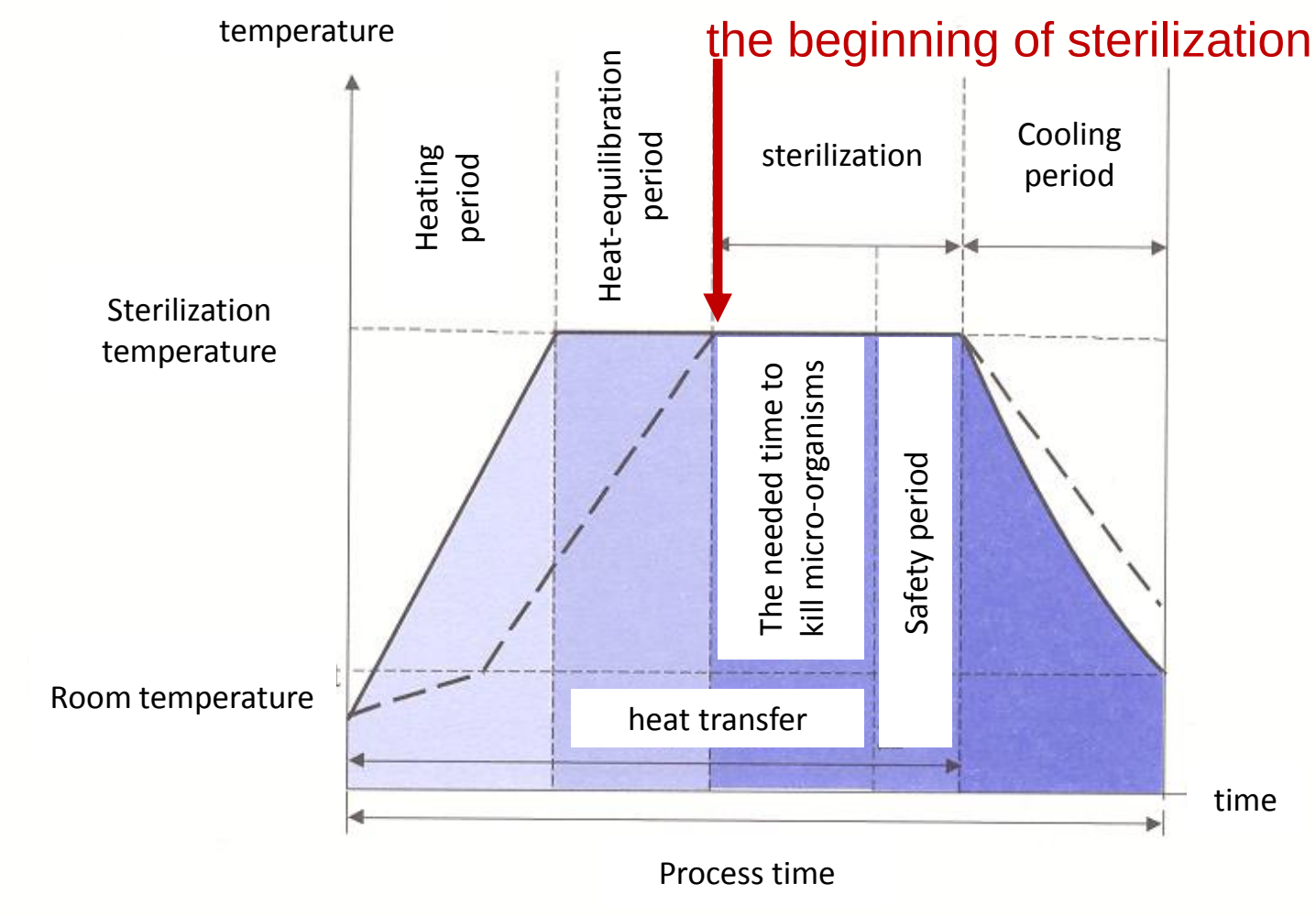
- Temperature-sensing elements on the coolest part of loaded sterilizer.
- Biological indicators

above 220°C ➔ sterilization + depyrogenation

Registration of pressure and temperature
Application of microbial indicators
Above 220°C sterilization of glasswares and depyrogenation

Dry heat sterilization

Procedure of dry-heat sterilization



Steam sterilization (heating in an autoclave)

Ph.Hg.VII

Autoclave		
°C	atm	min
134 ±2	+2.1	10
121 ±3	+1.1	20

Ph.Eur. 6:

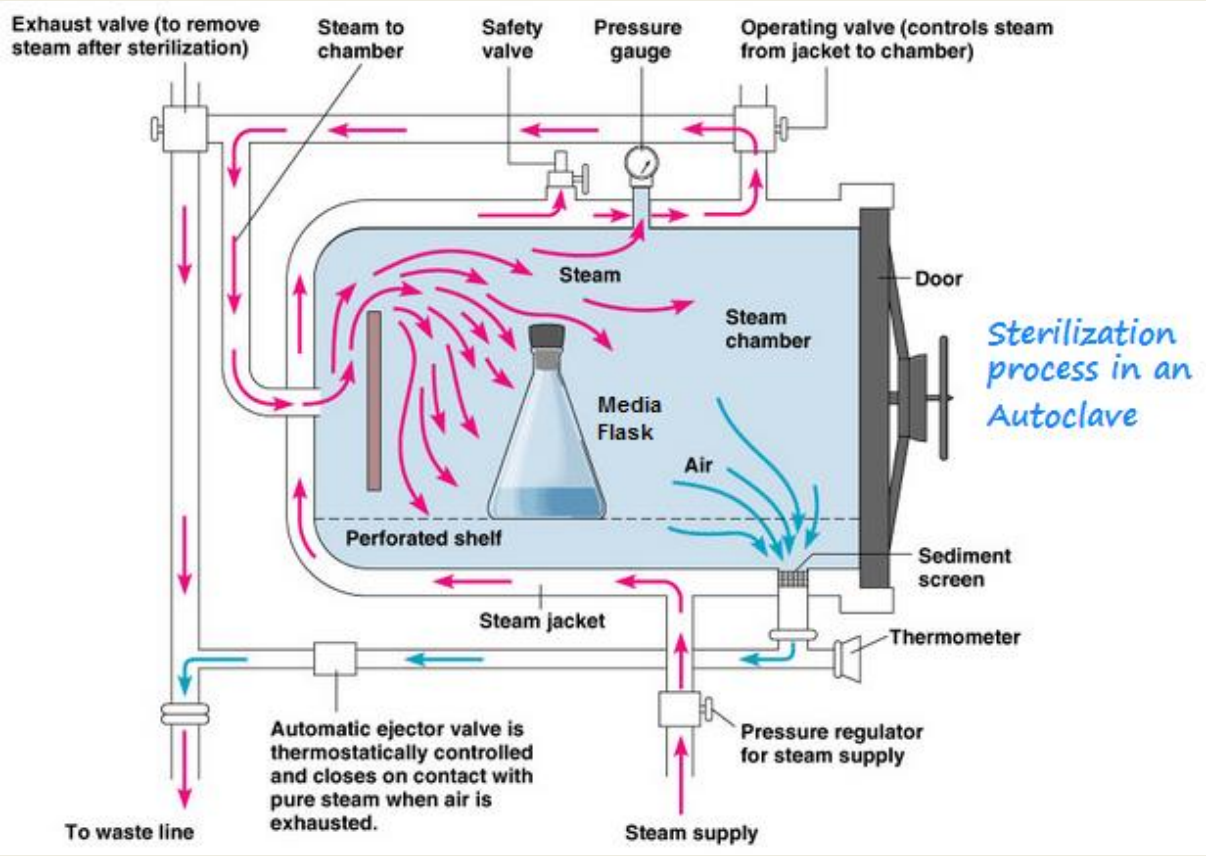
Under pressure.
Applicable, especially for aqueous preparations.(min: 15min, ≥125°C)

SAL 10^{-6} or better

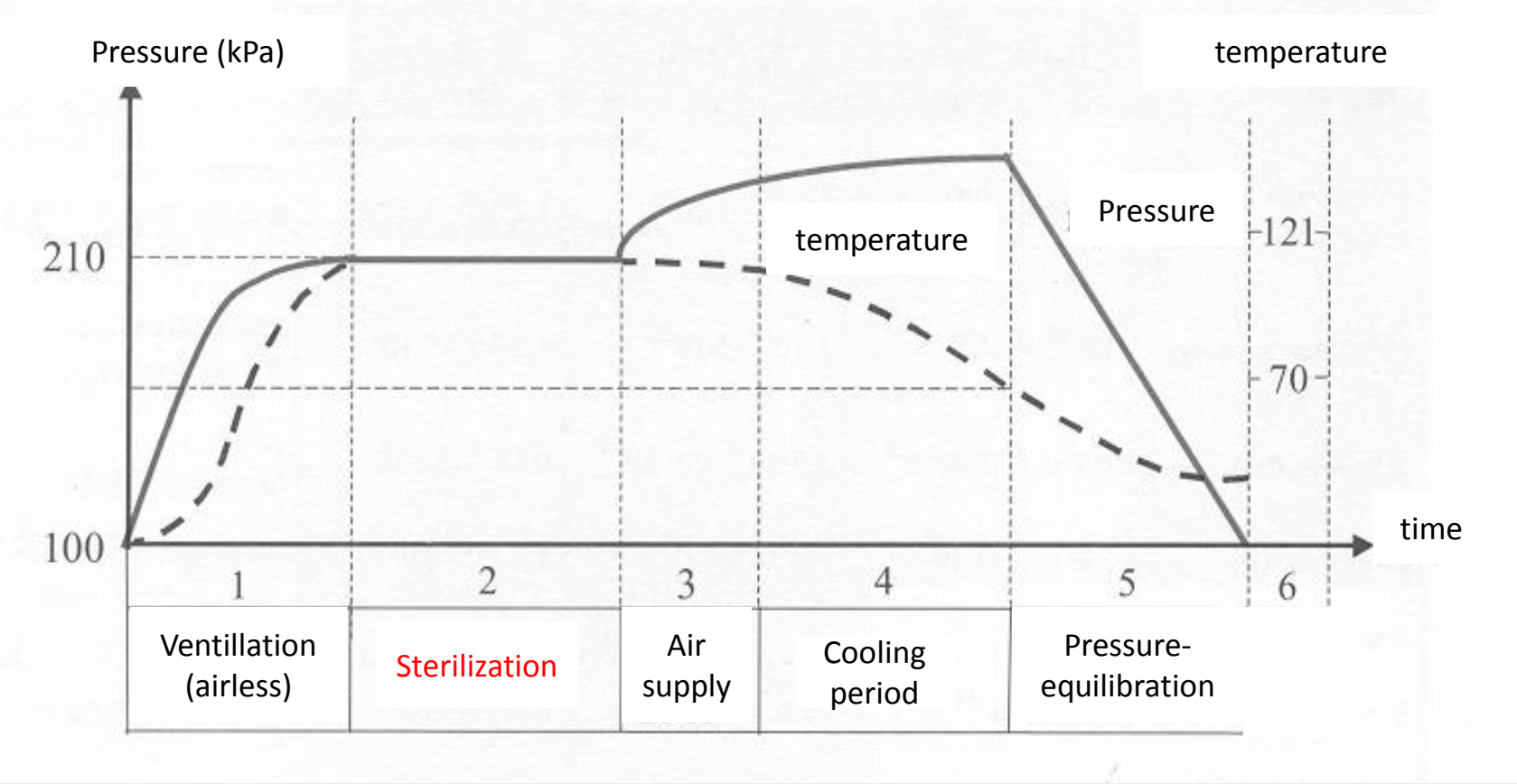
Registration of temperature and pressure

Microbial indicators

Steam sterilization (heating in an autoclave)



Steam sterilization (heating in an autoclave)



Radiation

Non-ionising radiation

Ultraviolet (UV)

- 200-400nm
- The most effective range: 240-280nm (germicide:253,7 nm)
- Aseptic work area, and processing
- Manipulator, lamina(i)r box
- Disadvantages: the glass absorbs the radiated beam (surface effect)

Infrared (IR)

- heat effect

Ionising radiation: **X-ray, α-, β-, γ-radiation**

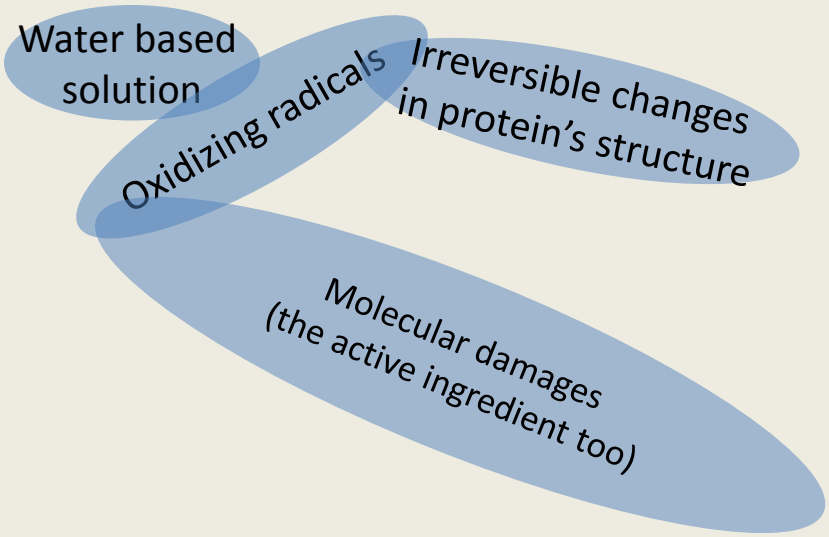
- Ph.Hg.VIII. (terminal sterilization)
- source: Co 60 or electrone-beam sterilizer

UV-radiation

Applied by aseptic work places. (germicide)

IR-radiation

The temperature of the system is increased.
The heat-sensitive microorganisms
will be damaged .



Radiation

Ionising radiation sterilization (Ph.Hg.VIII.:)

- ionising radiation
- radioisotropic source (cobalt 60) – gamma- or by electron accelerator
- standard: absorbed dose : 25 kGy (kgray=/J kg^{-1})
- SAL 10^{-6} or better
- dosimetric monitoring
- microbial indicators

Radiation

γ-radiating isotopes: (Ph.Hg.VII)

Cobalt-60 (^{60}Co) ➔ (^{58}Co in Ph.Eur 6, half time=70 days)

Half-time: 5.3 years

it is produced by ^{59}Co isotop with neutron-shooting

Eliminate β-radiation (> 0.3 MeV) and γ-radiation (2 photones) (1,17 and 1,33 MeV) ➔ stabile ^{60}Ni-isotop

Caesium-137 (^{137}Cs)

Half-time: 30 years

It is produced by uranium fission

1 γ-photone is eliminated (0.66 MeV)

Radiation

Lethal dose for different species

Species	Lethal dose ($kJ \cdot kg^{-1}$)
human	0.005 – 0.01
insects	0.01 – 0.25
bacteria	0.3 – 5.0
fungi	2.0 – 10.0
spores of bacteria	15.0 – 20.0
viruses	20.0 – 50.0

Filtration

Filtration: adsorption and sieve effect

Sintered-glass filter	Seitz-filters	Membrane filters
(G5): 1-1.5 μm	EKS-1: 1.0-1.2 μm	0.20-0.45 μm
sieve effect	asbestos + cellulose	cellulose polymers
	adsorption	sieve effect

Filtration

Filtration (Ph.Eur. 6):

- product that cannot be thermally sterilized
  - „microbial challenge test” (*passage trought the filter?)*

Preparation!!!

- *The location of filtering and filling must be near to each other.*
- 0.22 µm pore size or less
- The solutions do not absorb on the filters.
- Avoid the contaminants from the filter
- Stage of filter =„bubble point” (tested before and after use)

Attention is given to:

- **The bioburden prior to filtration**
- **Filter capacity**
- **Batch size**
- **Duration of filtration**

Filtration

Filtration (Ph.Eur. 6):

- **asbestos** fibers must be **eliminated**
- The **membrane filters** are made from **cellulose-nitrate** and -**acetate** or **polycarbonate**
- pore size of membrane filters= **0.20 µm**,
  in case of viscous solution= **0.45 µm**.
- If an eye drop is filtered, than the preparation must be **preserved**.

Filtration

Bacteria-retentive membranes

- Fast and great volume
    - Surface area
    - Pressure
    - Suction
- By infusions
    - **Pre-filtration**
        - Glass-fiber filter
    - **Depyrogenation**
        - Activated carbon
    - **Bacteria-retentive memberns:**
        - Cellulose polymers (CA,CN)
        - 0.45 or 0.22 μm

Gas sterilization

- Is only to be used where there is **no suitable alternative**
- Essential the penetration of gas and moisture into the materia
- End of the process: **residuals should not remain**
- Concentration, humidity, temperature, duration (register!)
- Microbial indicators
- Sterility test must be made from each batches

Gas sterilization

Chemical agents: if the materia is heat-sensitive

formaldehyde, β-propiolactone, ethylen-oxyde

Equipments and textiles

Drawback:
- Low penetration
- Odour, toxic
- Heavy removing (steam-rinsing)
- Polymerisation-coagulation

Non-irritable
Less-volatile
Effective in low concentration

Drawback:
- Penetration into poruous materia is bad
- Above 50°C polymerisation.

Effective
Good penetration capability,
Effective in room-temperature

Drawback:
- It is used in the suitable gas mixture
- The moisture of the materia can reduce its effectiveness.

Plasm sterilization

With hydrogen-peroxide:

Vacuumed workplace – spray the hydrogen-peroxide – irradiate with radio-wave (electromagnetic) – cold-plasm state (ions, free radicals)

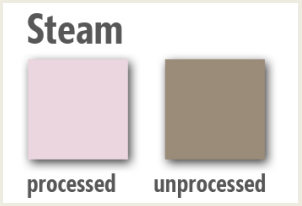
- medical equipment, metal tools, hand tattoo equipment, radiationtherapy devices, ultrasound heads
- Warning: cellulose, paper, liquids, powders, (vacuum)

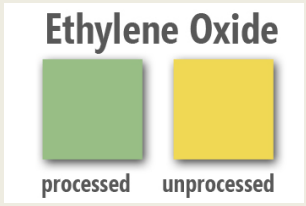
- Temperature of sterilization: 46±4 °C
- Ventillation: trought HEPA-filter (pressure equalization)
- Cyclical period: 28 min – 1 hour

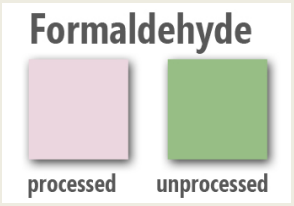
Indicators: physico-chemical / biological

Indicators

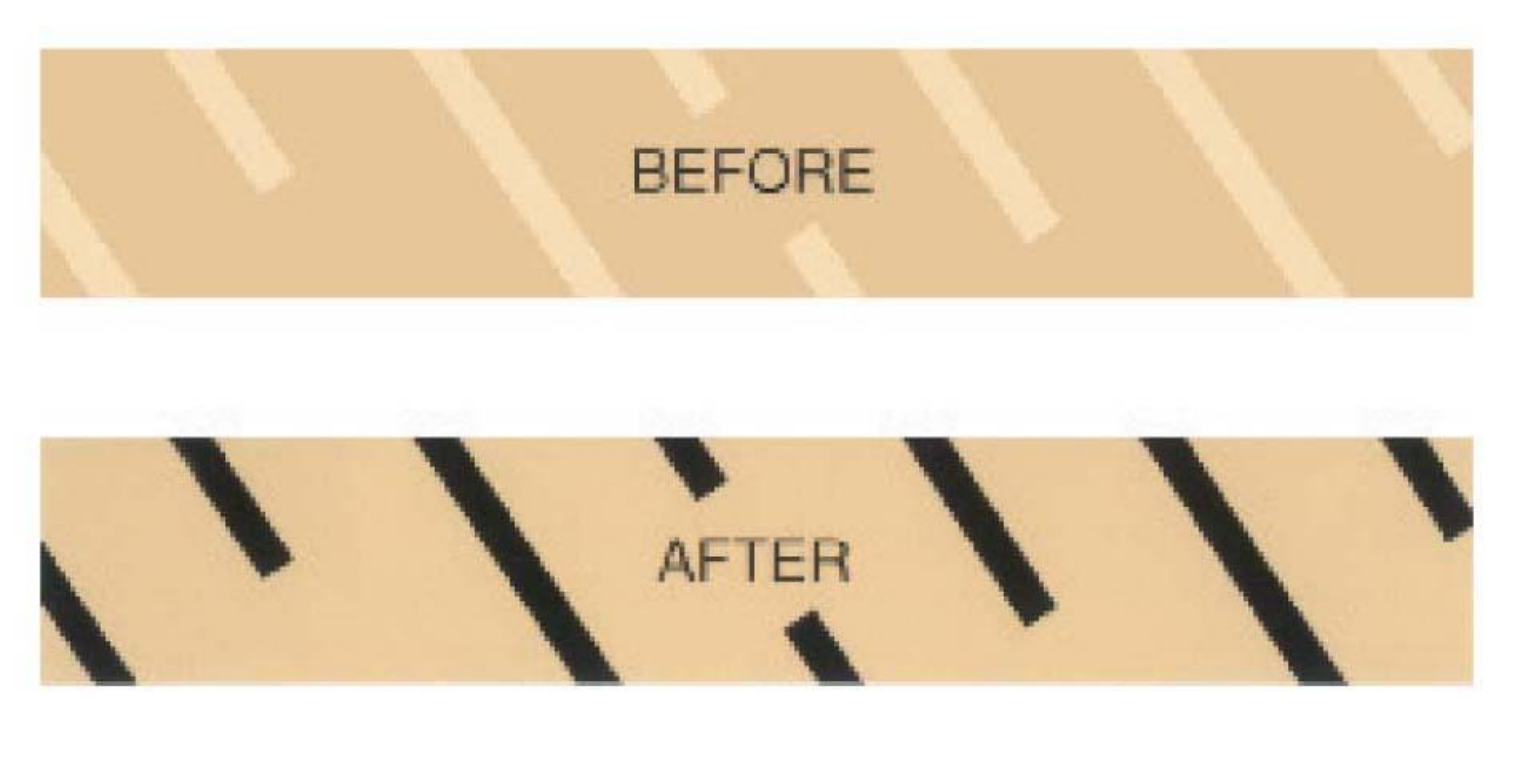
- **Indicator tape**
- **Indicator strips**
  (non-uniform color = air in the workspace
  not colored spots – overheated steam*)*
- **Brown's indicator tube**
- **Bowie Dick indicator pack**
  (appearence of: ventilation, air infiltration, influx of non-condensable gases into the steam)







Heat indicator



Biological indicators of sterilization (Ph. Eur. 6)

- „**Biological indicators are standardized preparations of selected micro-organisms used to assess the effectivenes of a sterilization procedure**.”
- „They usually consist of a population of bacterial spores placed on an inert carrier, for example a strip of filter paper, a glass slide or a plastic tube.”
- „may be inoculated directly into a liquid product to be sterilized.”

Biological indicators

– Name of the species of the bacterium

– The number of the strain in the original collection

– Number of viable spores per carrier

– D-value (value of a parameter of sterilization required to reduce the number of viable organisms to 10 per cent of the original number)

–„The resistance of the test strain to the particular sterilization method is great compared to the resistance of all pathogenic micro-organisms and to that of micro-organisms potentially contaminating the product.”

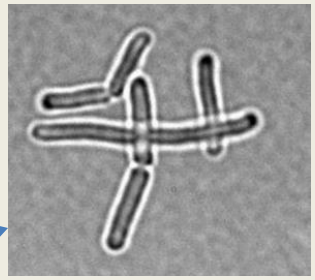
- „The test strain is non-pathogenic”
- „The test strain is easy to culture”

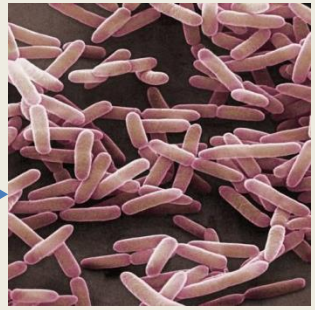
Biological indicators

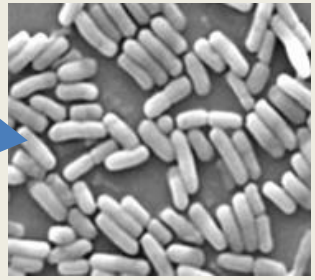
- „It is recommended that the indicator organisms are placed at the locations presumed, or wherever possible, found by previous physical measurment to be least accessible to the sterilizing agent.”
- „After exposure to the sterilizing agent, aseptic technique is used to transfer carriers of spores to the culture media, so that no contamination is present at the time of examination.”
- „After incubation, growth of the reference micro-organisms subjected to a sterilization procedure demonstrates that the procedure has been unsatisfactory.”
- Species:
  - *Bacillus stearothermophylus*
  - *Bacullus subtilis*
  - *Bacillus pumilus*

Biological indicators

Methods	Micro-organisms
Steam	B. Stearothermophilus
Dry heat	B. Subtilis var niger
Gas	B. Subtilis var niger
Radiation	B. pumilus







Aseptic compounding and dispensing

- „The objective of aseptic processing is to maintain the sterility of product that assembled from components.” (Ph.Eur. 6)

- **Aseptic processing:** the whole of those work methods and processes – including the person’s behavior and health status – which can keep away the microorganisms.

- „This is achieved by using conditions and facilities designed to prevent microbial contamination.”

Aseptic preparation

Careful attention:

- environment
- personnel
- critical surface
- container/closure sterilization and transfer procedure
- maximal holding period of the product before filling into the final container

Aseptic preparation

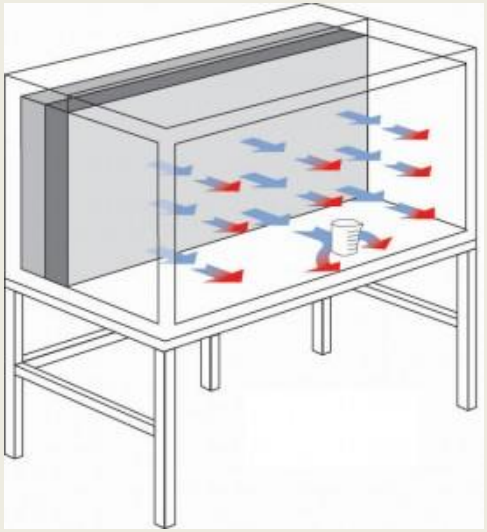
Appropriate concentation and time (with new solution)

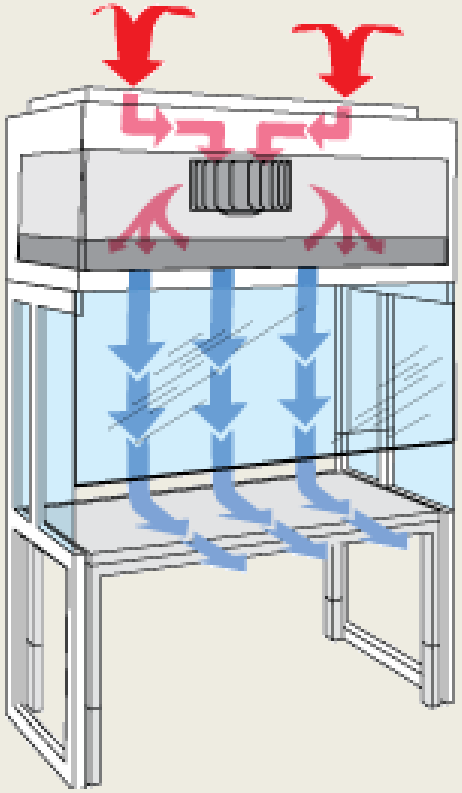
Aseptic preparation

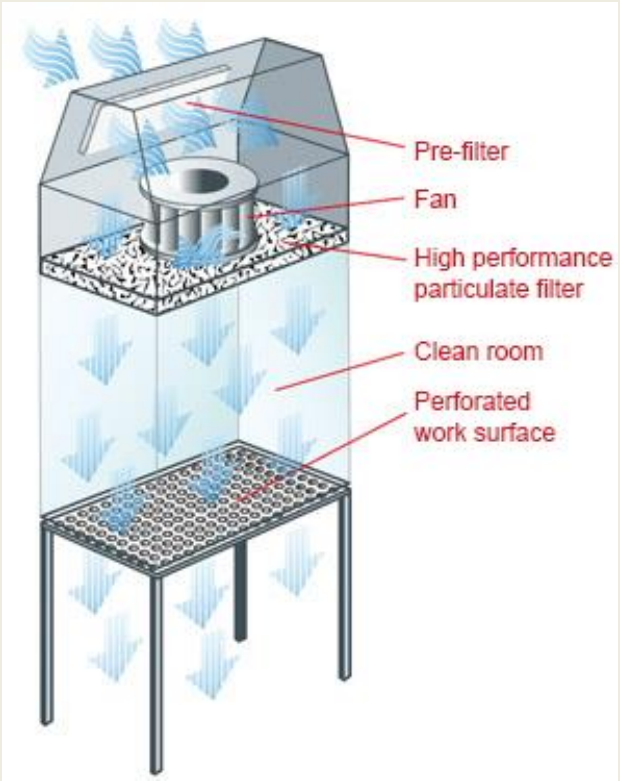
Aseptic preparation

Manipulator UV-radiation (Germicide)

Laminar box






Pre-filter
Fan
High performance particulate filter
Clean room
Perforated work surface


V MAT

Aseptic preparation

Infusion bottle

rubber stoppers

-cleaning
-rinsing with distilled water
-sterilizing

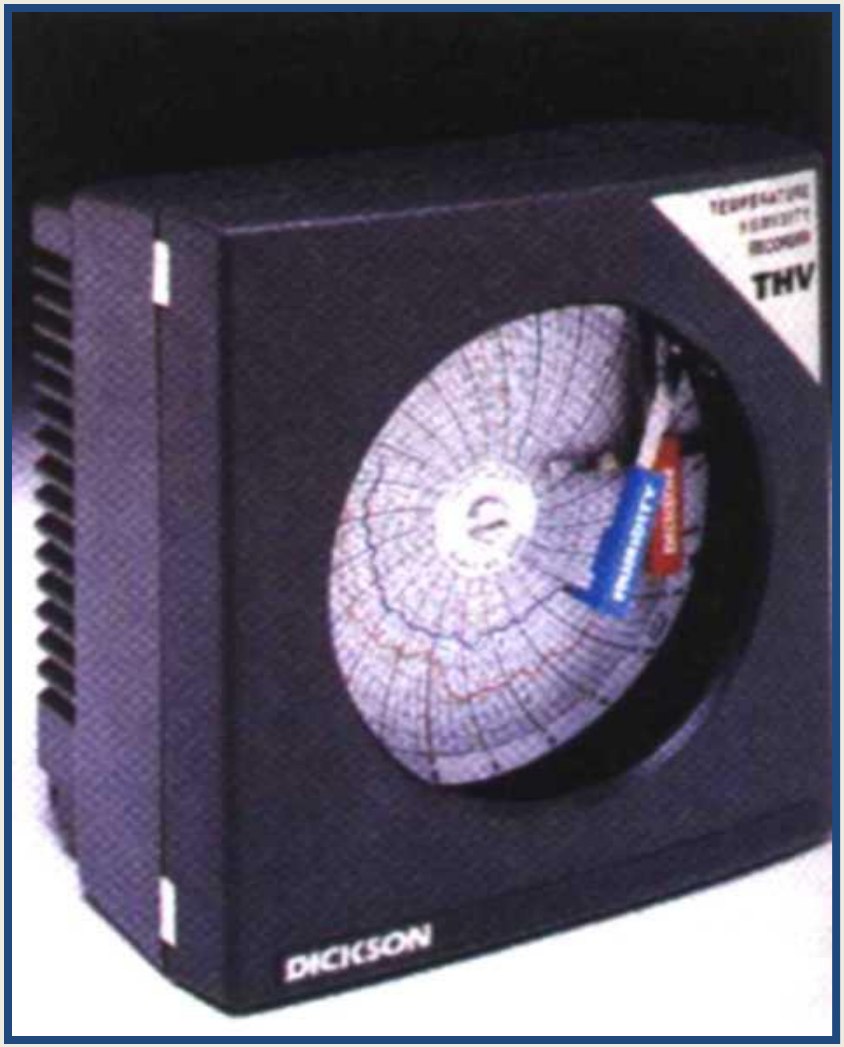
Sterilization

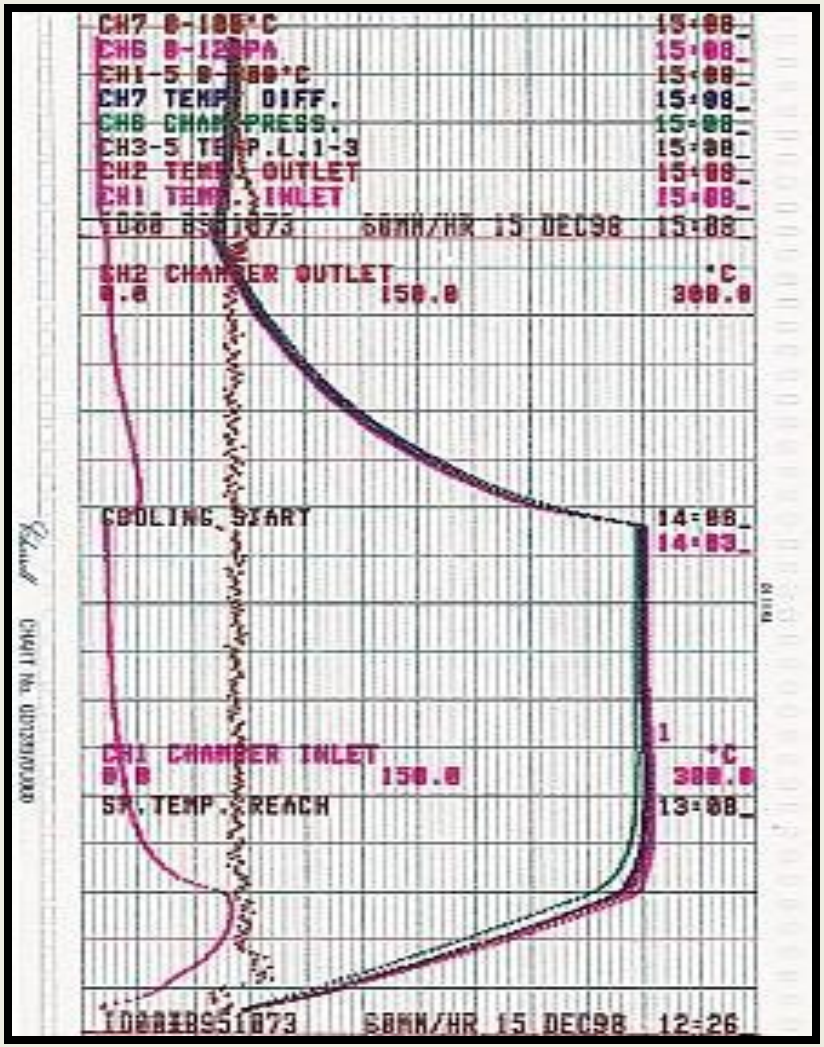
Controlling:

pressure
temperature
indicator
-chemical
-microbiological

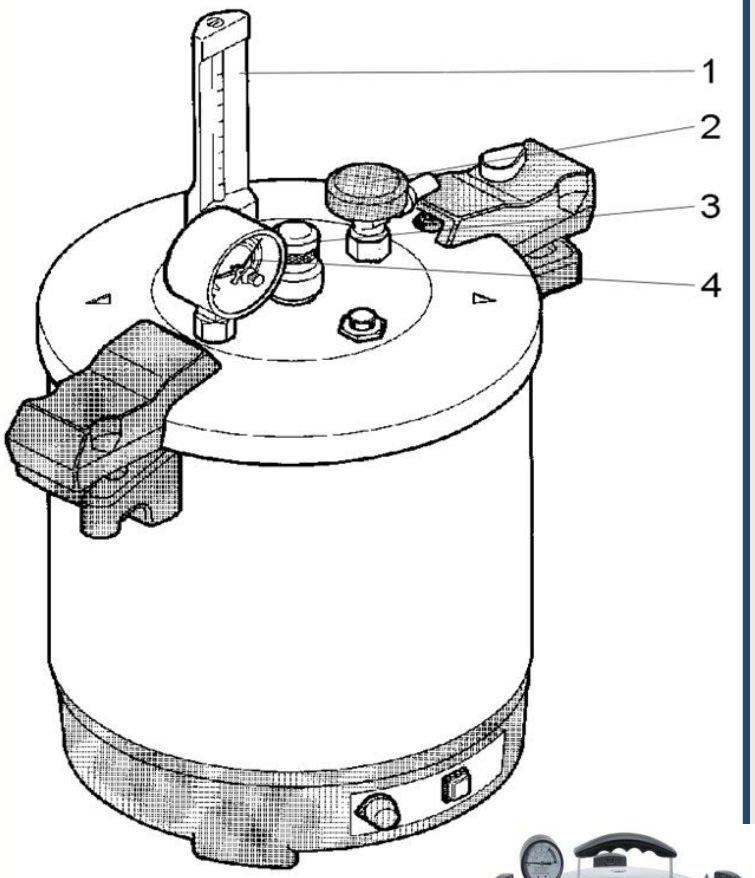
Sterilization

Process contolling



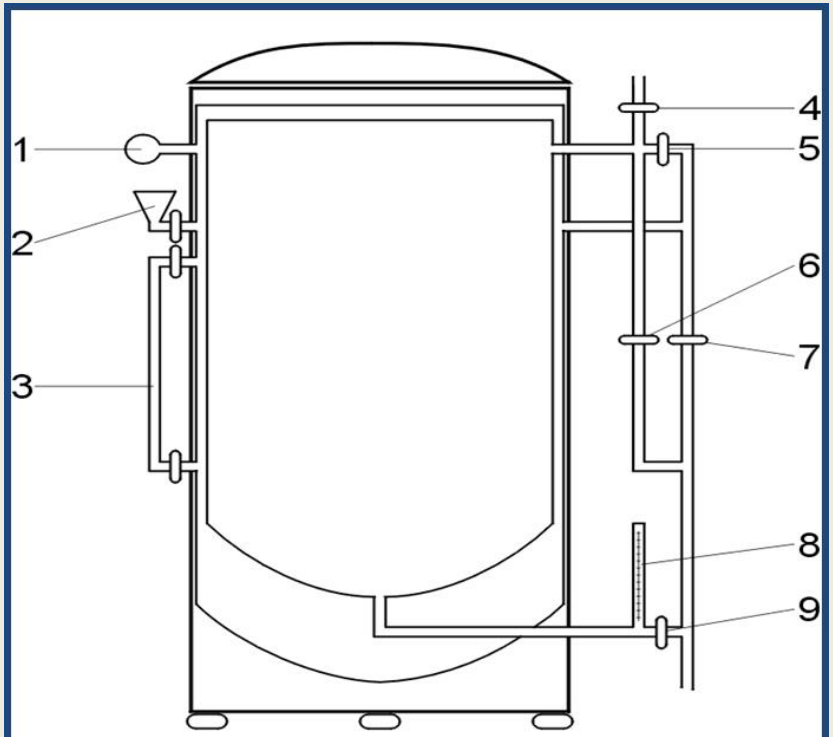


Certoclave and autoclave



Certokláv

1. Thermometer
2. Steam drain tap
3. Pressure control valve
4. Pressure gauge



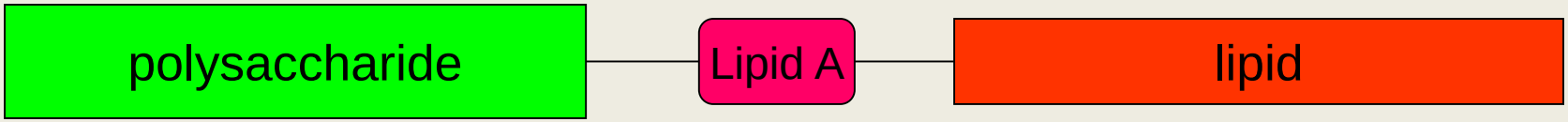
Autokláv

1. Ventillation valve
2. Water inlet
3. Water level indicator
4. Air suction tap
5. Steam inlet tap
6. Vacuum valve
7. Vacuum valve
8. Thermometer
9. Condensation tap

Depyrogenation

- **Pyrogens: materials, that can cause fever** (endo-, exogen), coagulation disorders and metabolic changes.

- Endotoxins of Gram-negative bacterias (lipopolysaccharide (LPS)) G-80%, G+20%,
- It binds to the external membrane
- When the bacterium dies, LPSs can dissolve into the media.
- Pyrogens must not be included in parenteral (>50ml) preparations.



Biological effect of endotoxins:

- fever
- coagulation
- metabolic changes

Depyrogenation

- Pyrogen’s chemical resistance is very high
- 200-250°C temperature is needed to destroy them
- Sensitive to oxydation
- It can be adsorbed by adsorbents:
    - **active carbon**
    - asbestos-filter

Examinations to pyrogens (Ph.Eur.6)

In vitro: **LAL-test** (**L**imulus **A**moebocyta **L**isatum)

„The test for bacterial endotoxins is used to detect or quantify endotoxins of G- bacterial origin using amoebocyta lysate from horseshoe crab (Limulus polyphemus).”

- gel-clot technique (based on gel formation)
- Turbidimetric technique (based on the development of turbidity after cleavage of an endogenous substrate)
- Chromogenic technique (based on the development of colour after cleavage of synthetic peptic-chromogen complex.)

They may be kinetic, semi-quantitative or end-point methods.

In vivo: **rabbit** pyrogen test

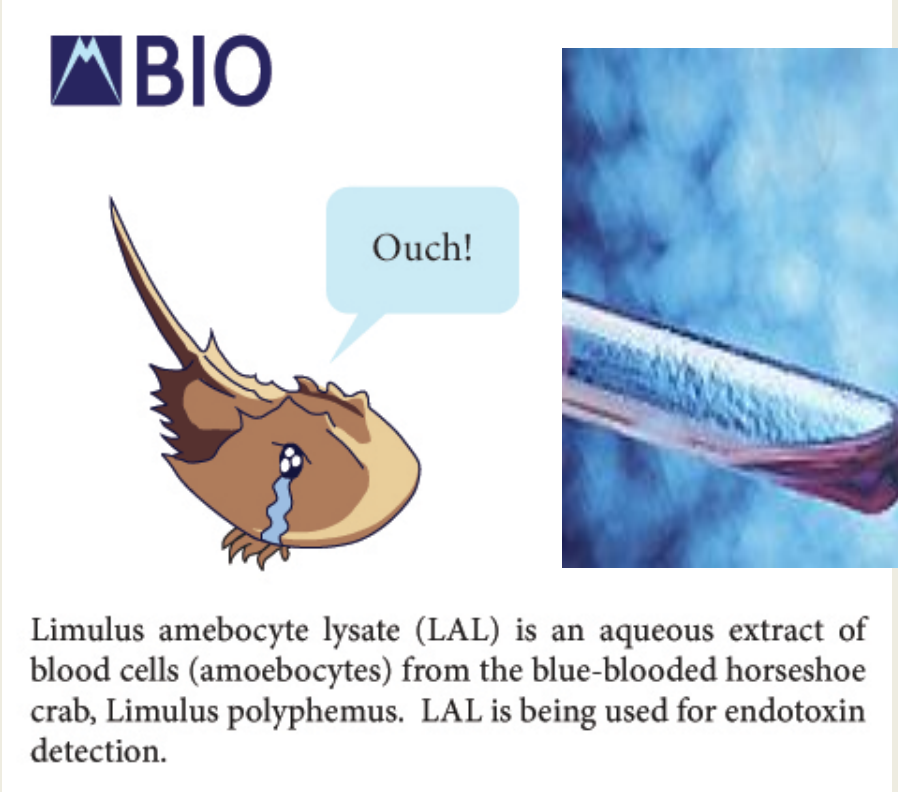
Inserted into veins of rabit’s ears.

Detecting the body temperature.

LAL-test

Advantage:

- In vitro process
- fast, duration: 1.5 h
- sensitivity is good
- cheap



Limulus amebocyte lysate (LAL) is an aqueous extract of blood cells (amoebocytes) from the blue-blooded horseshoe crab, Limulus polyphemus. LAL is being used for endotoxin detection.

Disadvantage:

- just G -, (no virus)
- interaction with

(HCO_3, phosphates, citrates, glucose)

- It is water based test. (oil based hormones!!)
- inorganic materials (rubbers, closures) are not measurable.

Sterile preparations

- Injections
- Infusions
- Single dose eye drops, eye ointments
- Powders for tablets for parenteral administration
- Implants
- Enteral and parenteral nutrition
- Haemodialysis solutions
- Aerosols for inhalation and wound surfaces
- Other dosage forms (wound ointment / gel, wound sprinkling powder)

Ph.EUR. (Ph.Hg.VIII.)

Micro-biological class	Preparations	Requirements
1.	Sterilization is required by the appropriate dosage form and sterile according to the label of other preparations	**Sterility**

Ph.EUR. (Ph.Hg.VIII.)

Micro-biological class	Preparations	Requirements
2.	Topical and respiratory pharmaceutical preparations, except where sterility is required Transdermal patches (Tests both adhesive and substrate)	all viable 10^2 aerobic bacteria and fungi / g or ml -allowed: 10^1 enterobacteriaceae and other gram-negative bacteria / g or ml - except: not for transdermal patches! excluded: Pseudomonas aeruginosa (1.0 g or 1.0 ml) Staphylococcus aureus (1.0 g or 1.0 ml)

Ph.EUR. (Ph.Hg.VIII.)

Micro-biological class	Preparations	Requirements
3.	A. / Pharmaceuticals for oral and rectal administration B. / Oral preparations of natural (animal, plant, mineral) origin, for which antimicrobial pre-treatment is not possible and whose starting material is 103 viable micro-organisms / g or ml from the competent authority. Herbs included in microbiological class 4 are excluded.	Total viable aerobic count 10^3 bacteria/g or ml and 10^2 fungi/g or ml Excluded: Escherichia coli (1.0 g or 1.0 ml) Total viable aerobic micro-organism count 10^4 aerobic bacteria/g and ml 10^2 fungi/g or ml Not more than 10^2 enterobacteriaceae and other gram-negative bacteria / g or ml excluded: Salmonella (10.0 g or 10.0 ml) Escherichia coli (1.0 g or 1.0 ml) Staphylococcus aureus (1.0 g)

Ph.EUR. (Ph.Hg.VIII.)

Micro-biological class	Preparations	Requirements
4.	This class includes herbal preparations containing exclusively herbal, whole, comminuted or powdered drug (s). A ./ Herbal preparations that need to be boiled before use. B. / Herbal preparations that do not require scalding before use.	Total viable aerobic micro-organism count: 10^7 aerobic bacteria/g or ml and 10^5 fungi/g or ml Not more than 10 Escherichia coli/g or ml Total viable aerobic count 10^5 aerobic bacteria/g or ml and 10^4 fungi/g or ml Not more than 10^3 Enterobacteriaceae and other gram-negative bacteria/g or ml Excluded: Escherichia coli (1.0 g) Salmonella (10.0 g)

Thank you
for your attention!