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Sterilization requirements – a regulatory view

Dr. Jan Havel

MHS – Gate to worldwide markets

Full support world wide

conformity assessment & testing

Canada
MDSAP (ISO 13485), CAN/CSA C22.2 NR.601.1 as NRTL

Europe
Conformity assessment procedures according to MDR, IVDR, AIMDD, MDD, IVDD (notified body number 0123)

Russia
Certification via GOST

Taiwan
Technical Cooperation Program

USA
FDA 510(k) NRTL, FDA QSReg inspection in compliance with MRA

Japan
Recognized Certification Body (RCB), Medical Test Lab, certification (PAL, RCB)

China
SFDA Registration, CCC (China Compulsory Certification)

Brazil
Product certification via agreements with UCIEE or CERTUSP

Australia
Certification as CAB under MRA (Conformity Assessment Body)

New:
- Biocomp Lab
- Saudi Arabia CAB
- Consulting Japan
- Korea CAB

General requirements Directive 93/42/EEC (MDD)

- Sterilization: Annex I of MDD
  - *8.3. Devices delivered in a sterile state must be designed, manufactured and packed in a non-reusable pack and/or according to appropriate procedures to ensure that they are sterile when placed on the market and remain sterile, under the storage and transport conditions laid down, until the protective packaging is damaged or opened.*
  - *8.4. Devices delivered in a sterile state must have been manufactured and sterilized by an appropriate, validated method.*
  - *8.5. Devices intended to be sterilized must be manufactured in appropriately controlled (e. g. environmental) conditions.*
  - *8.6. Packaging systems for non-sterile devices must keep the product without deterioration at the level of cleanliness stipulated and, if the devices are to be sterilized prior to use, minimize the risk of microbial contamination; the packaging system must be suitable taking account of the method of sterilization indicated by the manufacturer.*
  - *13.6 (h) if the device is reusable, information on the appropriate processes to allow reuse, including cleaning, disinfection, packaging and, where appropriate, the method of sterilization of the device to be resterilized, and any restriction on the number of reuses.*

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Bioburden estimation EN ISO 11737-1

Bioburden

- Germ contamination - Bioburden: (determined based on EN ISO 11737)
- „population of viable microorganisms on or in product and/or sterile barrier system“

Influencing parameters

- Initial level of product contamination (= bioburden)
  Sources:
  - Raw materials
  - Used components
  - Manufacturing process
  - Environmental conditions during production
  - …
- Packaging of products
- Sterilization method
- Storage, transport, handling

Where are microorganisms in the production?

- Particle:about 1 organism / 1000 micro particles
- Therefore sources like:
  - Clothes: e.g. shoes
  - Raw materials
  - Cleaning equipment: mop, wiper, etc.
  - Media: liquids, gases, etc.

  Shall be monitored during production of sterile products/aseptic production (cleanroom).

To be considered for a suitable sample

- The product shall be in the final packaging
- Product family:
  - Number of microorganisms
  - Sort of microorganisms
  - Product size/part and their number
  - Complexity of the product
  - Level of manufacturing automation
  - Product environment

EN ISO 11737-1 A.5.1.2

What is the consequence for manufacturing of sterile medical devices?

- If microorganisms are enabled to grow in the manufacturing environment, they will be found on the medical device!
  - The extent and the nature of the microbial bioburden has to be known before sterilization:
    - Manufacturing has to be performed in a clean environment
    - The product has to be decontaminated –if necessary- prior to sterilization.
  - Products have to be protected from contamination by choice of a suitable packaging material.
  - The bioburden reflects the influence of the complete production process and control measures.

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Contamination control

Cleanrooms – Why?

Would you manufacture a medical device in here?

Cleanrooms – Why?

- Sterilization processes are limited in their capability to kill microorganisms.
  Consequence:
  - Organisms with a high resistance to the sterilization process may survive
  - Organic residues of contamination may remain on the products:
    - Toxins
    - Endotoxins
    - Discolourations
- Particles cannot be removed from the product via sterilization.
  - There is clear guidance for particulate contamination on medical devices.
    Possible consequences:
    - Bioincompatibility might develop
    - Particles might migrate into the body and cause severe damages
    - Rejection (of implants) might be induced
- Cross-contamination of equipment and product shall be prevented!

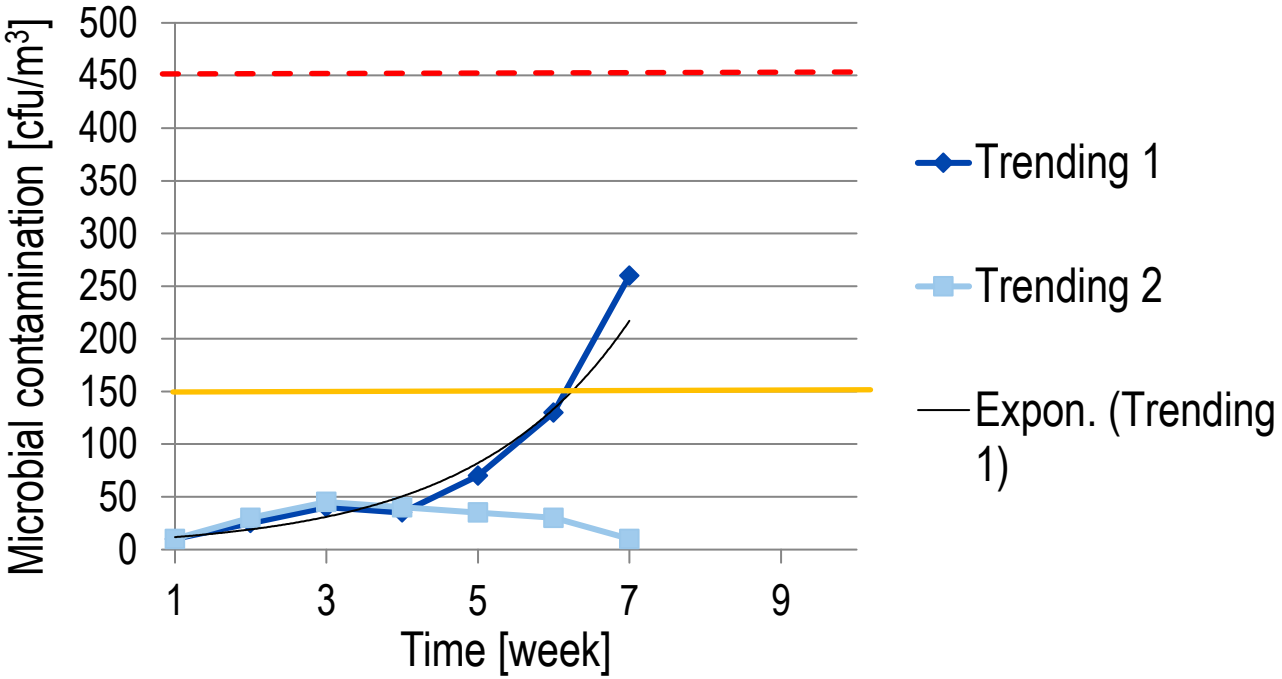
→ Contamination control is therefore of significant importance for medical devices and sterile products

Regulative requirements

- Medical device directive (MDD) Annex I 8.5:
  - Devices intended to be sterilized must be manufactured in appropriately controlled (e. g. environmental) conditions.
- EU-GMP/Annex1:
  - The manufacture of sterile products should be carried out in clean areas entry to which should be through airlocks for personnel and/or for equipment and materials. Clean areas should be maintained to an appropriate cleanliness standard and supplied with air which has passed through filters of an appropriate efficiency.
- EN ISO 13485/6.4:
  - Documented requirements to the cleanliness (personnel, environment, product) have to be fulfilled.
- EN ISO 14644 Series: Cleanroom classification, test methods, monitoring, construction
- EN ISO 14698-1 -2/ EN 17141: Biocontamination control

Definitions

- **Cleanroom monitoring programm:**
  - A defined, documented programm includes:
    - Routine monitoring of the particle number and microbiological contaminations with analysis of trends.
    - Definition of limits
    - Regulation in case of a violation of the limits



MDD Annex I 8 EN ISO 14644-1 2.1.1 EN ISO 14664-5 A.3 EN ISO 14698-1 4 5.2 5.3 + -2 EN ISO 11737-2 8.5 8.6 21 CFR 211.42bc, 46bc, 63, 65a, 67a, 113b

Classification of cleanrooms

- A convention for classification was defined based on the measurable particle sizes.

ISO-Classification number (N)	(particle per m³ air)					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
ISO-class 1	10	-	-	-	-	-
ISO-class 2	100	24	10	-	-	-
ISO-class 3	1 000	237	102	35	-	-
ISO-class 4	10 000	2 370	1 020	352	83	-
ISO-class 5	100 000	23 700	10 200	3 520	832	-
ISO-class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO-class 7	-	-	-	352 000	83 200	2 930
ISO-class 8	-	-	-	3 520 000	832 000	29 300
ISO-class 9	-	-	-	35 200 000	8 320 000	293 000

EN ISO 14644-1 Table 1

Alert level for cleanroom monitoring

- **Alert Level:**

Defined microbial and particulate value which provides an early warning for a non typical drift of the contamination over time under normal manufacturing conditions (e.g. trending analysis).

  - Has to be orientated on historic data
  - Has to provide enough space (adequate lead time) for reaction
  - Is the basis for root cause analyses and possible actions

- **Action Level:**

Defined microbial and particulate value which results in case of excess immediate action and corrective actions.

  - Definite hazard for product and patient is given

ISO 14698-1 5.2 ISO 14698-2 EN ISO 14644-5 Annex EN ISO 11737-2 8.5 8.6

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Biological Effects

Biocompatibility Report content in general

What it a necessary documentation for a biocompatibility conformance assessment:

- Prepared by qualified persons
- With a structure based on a Risk management approach (EN ISO 14971)
  - *Not only a bundle of test reports!*
- Reference to other referring sections of the technical documentation possible
- Evidence for all claims needed
- Rigour in biological evaluation dependent on intended use and identified hazards

Report content

- Intended use
- Categorization according to EN ISO 10993-1
- Device description as relevant for BC
- Information on manufacturing, packaging, sterilization
- Material/Chemical characterization
- Identification of possible biological hazards
- Data review, risk analysis
- Testing & interpretation
- Toxicological risk assessment
- Statement on completion of risk analysis & control
- Overall biological safety assessment
- Conclusion

Biological Evaluation Report

Xxxxxxxxxxxxxxxxxxx
Xxxxx
Xxxxxxx xxxx xxxxx xx xxxx
Xxxxxx xxx x

Pyrogens in relation to sterile manufacturing

- Are substances which can cause:
  - Fever
  - Shock
  - Death
  - chemical/material based pyrogens (e.g. by particles)
  - Endotoxins (by bacterial residues):

Limits of entotoxin tests:

Medical devices in general	Medical products with cerebro-spinal contact
0.5 EU/ml	0.06 EU/ml
20 EU/device	2.15 EU/device

EN ISO 10993-11, Eu. Pharm.

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Cleaning and disinfection

Regulatory requirements

- EN ISO 15883: Cleaning- Disinfection equipment
  - Part 1: General requirements, terms and test methods
  - Part 2: Requirements and tests for thermal disinfection of instruments, anaesthesia equipment, bowls etc.
  - Part 3: Requirements and tests for thermal disinfection of containers for human excrement
  - Part 4: Requirements for tests of chemical disinfection for thermolabile endoscopes
  - Part 5: Test soils for proof of cleaning capability (non-harmonized standard)
- EN 14885: Disinfectants and their qualification
- EN ISO 17664: Information, which have to be provided by the manufacturer, for reprocessing of resterilizable medical devices
- Eu Pharm.:
  - Monographie 1167. Water for Dilution
  - 01/2005:0169 WFI. Water for Injection
  - 01/2005:0008. Water Purified
  - 01/2005:1927. Water highly purified

Why are certain products cleaned prior to sterilization?

- Sterilization processes are limited in their capability to kill microorganisms.
  Consequence:
  - Organisms with a high resistance to the sterilization process may survive
  - Organic residues of contamination may remain on the products:
    - Toxins
    - Endotoxins
    - Discolourations
- Particles cannot be removed from the product via sterilization.
  - There is clear guidance for particulate contamination on medical devices.
    Possible consequences:
    - Bioincompatibility might develop
- Production aids might remain on the products:
  - Oils, fats
  - Chemicals
- Some materials contain a too heavy initial bioburden at incoming.

Validation documentation for cleaning/disinfection

- Risk assement for each step of the reprocessing cycle (EN ISO 14971)
- Validation protocol for cleaning/disinfection
  - Defined cleaning/disinfection process (exposure times, temperature, concentrations, etc.)
  - Acceptance criteria for residual contamination:
    - Chemicals – only for chemical process
    - If applicable: endotoxin
    - Residual bioburden
  - Equipment has to be maintained and calibrated (washer disinfecter unit (WDU) must be in compliance with EN ISO 15883)
  - Only clearly defined disinfectants (medical devices) with defined performance may be used
  - Requirement for product performance e.g.:
    - Biocompatibily
    - Corrosion
    - Performance tests for proof of a safe product
  - Frequency of revalidation
- Validation report of disinfection
  - Confirmation that all defined conditions of the protocol were met.

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Bioindicators EN ISO 11138

Regulatory background

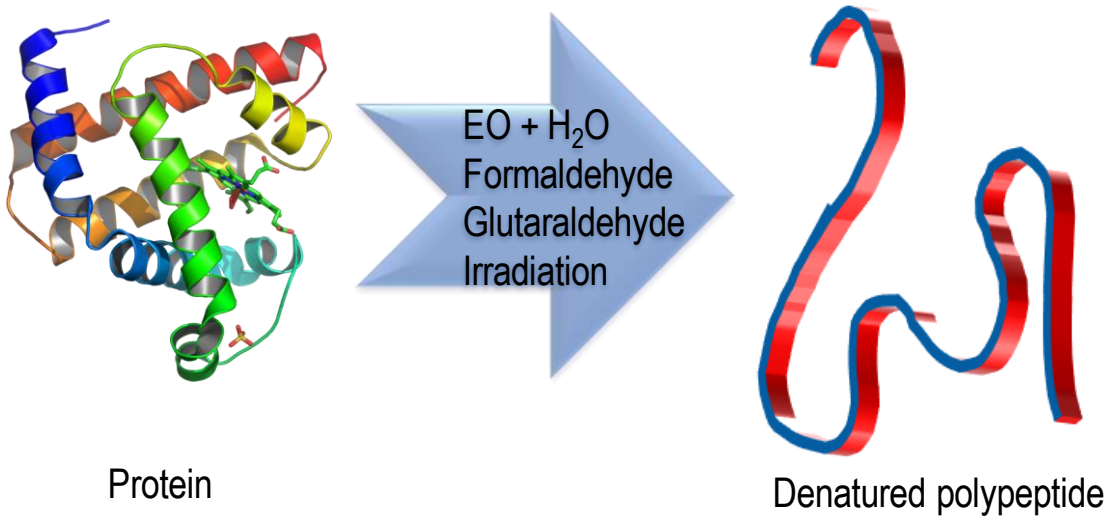
- EN ISO 11138: Biological indicators (BI)
  - 1 General requirements
  - 2 BI for ethylene oxide sterilization processes
  - 3 BI for moist heat sterilization processes
  - 4 BI for dry heat sterilization processes
  - 5 BI for low-temperature steam and formaldehyde sterilization processes
  - 6 BI for hydrogen peroxide vapour sterilization processes
  - 7 Guidance for the selection, use and interpretation of results

Typical incubation conditions

- EO
  - *B. atrophaeus*: with D-value ≥ 2,5 min at 54°C (EN ISO 11138-2):
    - 7 days
    - 30-35°C
- Moist heat
  - *G. staerothermophilus* or *Bacillus subtilis ATCC 35021* with a D-value ≥ 1,5 min and Z ≥ 6°C at 121°C (EN ISO 11138-3) :
    - 7 days
    - 55-60°C

Any deviations of the 7 days are perceived as reduced incubation time and needs an own validation thereof.

Sterilization



How can microorganisms be inactivated?

- Typical standardized methods:
  - Moist heat sterilization (Pasteurization, Disinfection)
  - Ethylene oxide
  - Irradiation (Gamma, e-beam, X-Ray, UV)

- Special methods:
  - Dry heat
  - Peroxide, oxygen radicals
  - Glutaraldehyde
  - Peracetate
  - Formaldehyde

When is which method typically favored?

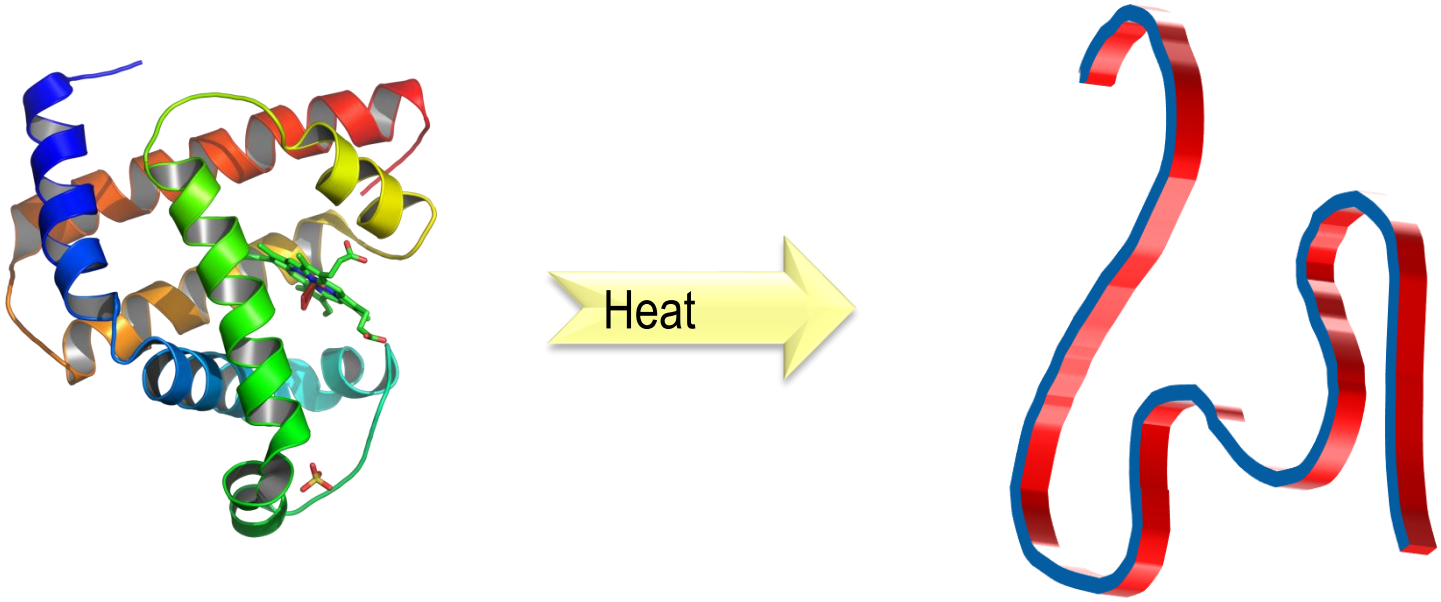
- Depends on the product:
  - If the product is thermal stable:
    - Moist heat sterilization:
      - No chemical residuals, no known resistances
      - Dry heat sterilization (Be careful: Non standard process)
  - Thermolabile Products:
    - Irradiation (z.B. gamma, X-ray, e-beam)
      - If the product does not change its performance via irradiation (e.g. PP)
      - If the product does not have to be cooled (X-ray and e-beam might heat it)
      - If the bioburden can be sterilized by irradiation
    - Ethylene oxide sterilization:
      - If above mentioned methods are not applicable
      - If the product is easily accessible for gas
      - If the product can withstand vacuum and humidity and does not react with EO
      - If the product/material contains good degassing characteristics
      - If the bioburden can be sterilized by EO

When is which method typically favoured?

- Depends on the product:
  - Chemical stable products / surface sterilization:
    - H_2O_2, peracetate sterilization
      - If aforementioned methods are not applicable (peracetate: Not suitable for metal)
      - Industrially rarely used for medical products (typically: in the aseptic for isolators)
  - If the product can withstand crosslinking chemical attacks
    - Glutaraldehyde
      - If the methods mentioned above are not applicable
      - If organic tissue has to be sterilized
      - If the product can withstand temperatures of up to 60°C
      - If the bioburden is not resistant
    - Formaldehyde (LTSF: Low Temperature Sterile Fluid)
      - Industrially rarely used
      - If the methods mentioned above are not applicable
      - If the product is easily accessible for gas
      - If the product can withstand vacuum and humidity and does not react with formaldehyde
      - If the product/material contains good degassing characteristics
      - If the bioburden can not be sterilized by EO
      - If the intended use permits it (cancerogen!)

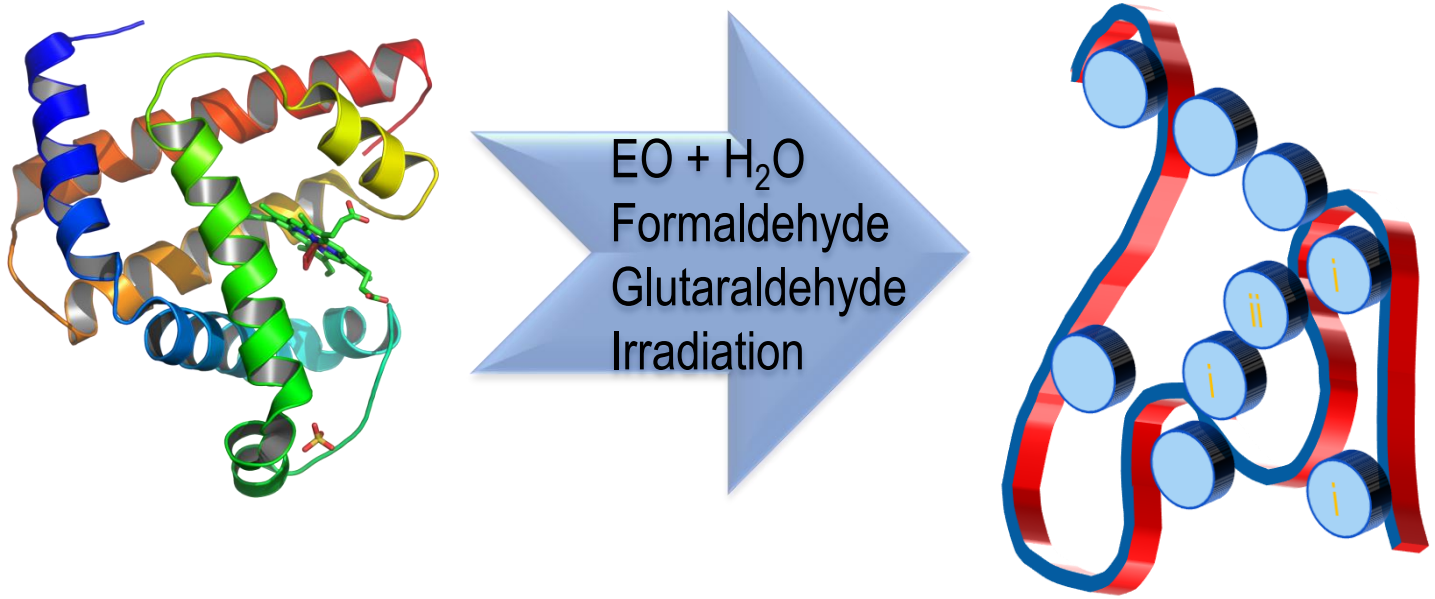
What happens during sterilization?

- Proteins are denatured or their functional structure is dissolved:
  - Thermal energy is denaturing proteins and dissolves chemical and physical bonds.



What happens during sterilization?

- Proteins are denatured or their functional structure is dissolved:
  - Thermal energy is denaturing proteins and dissolves chemical and physical bonds.
  - Intercalation / reaction with polypetides is denaturing proteins and breaks chemical and physical bonds.

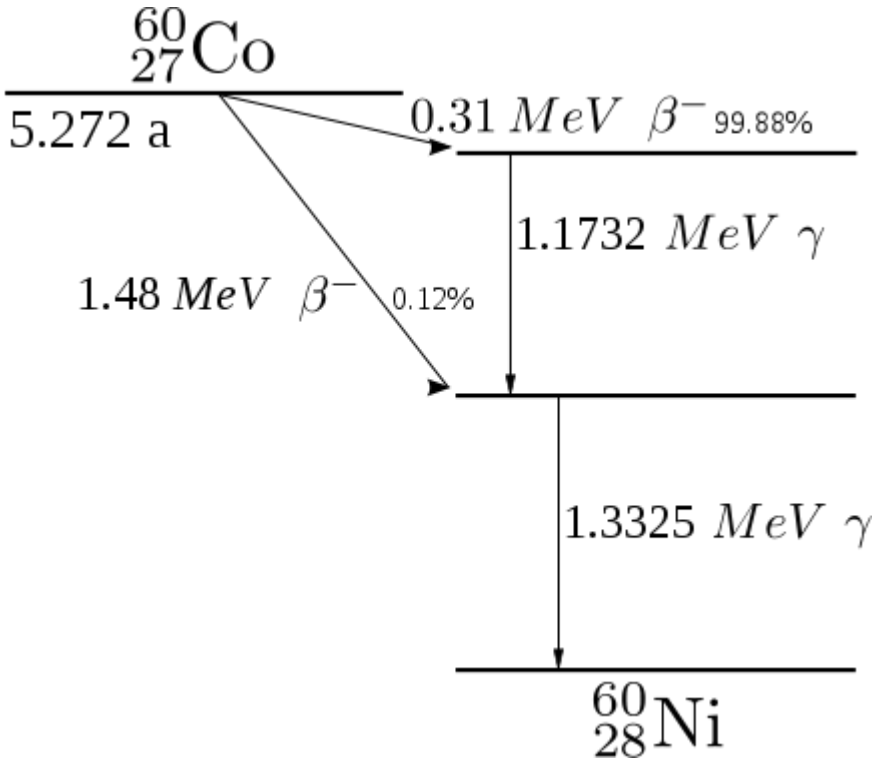


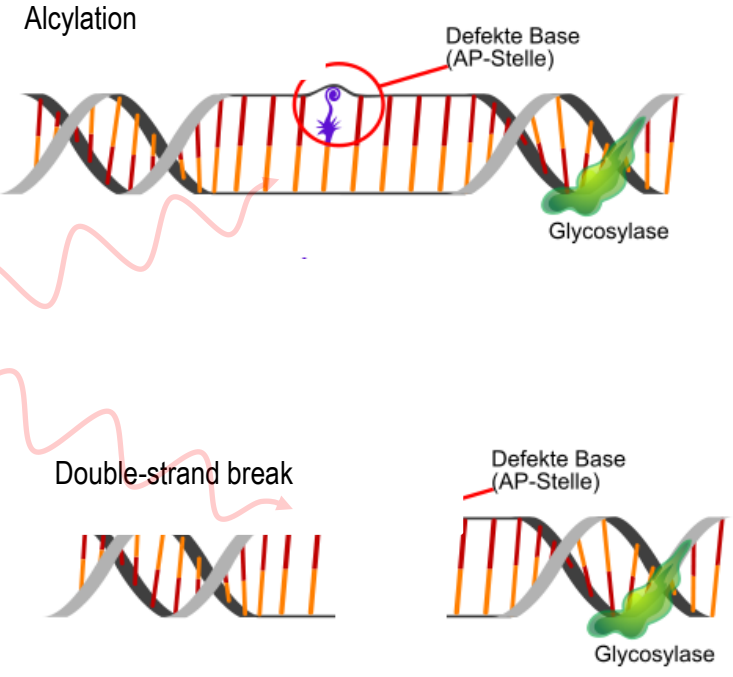
Protein

Denatured polypeptide

What happens during irradiation sterilization?

- DNA is destroyed or looses its function by modification.



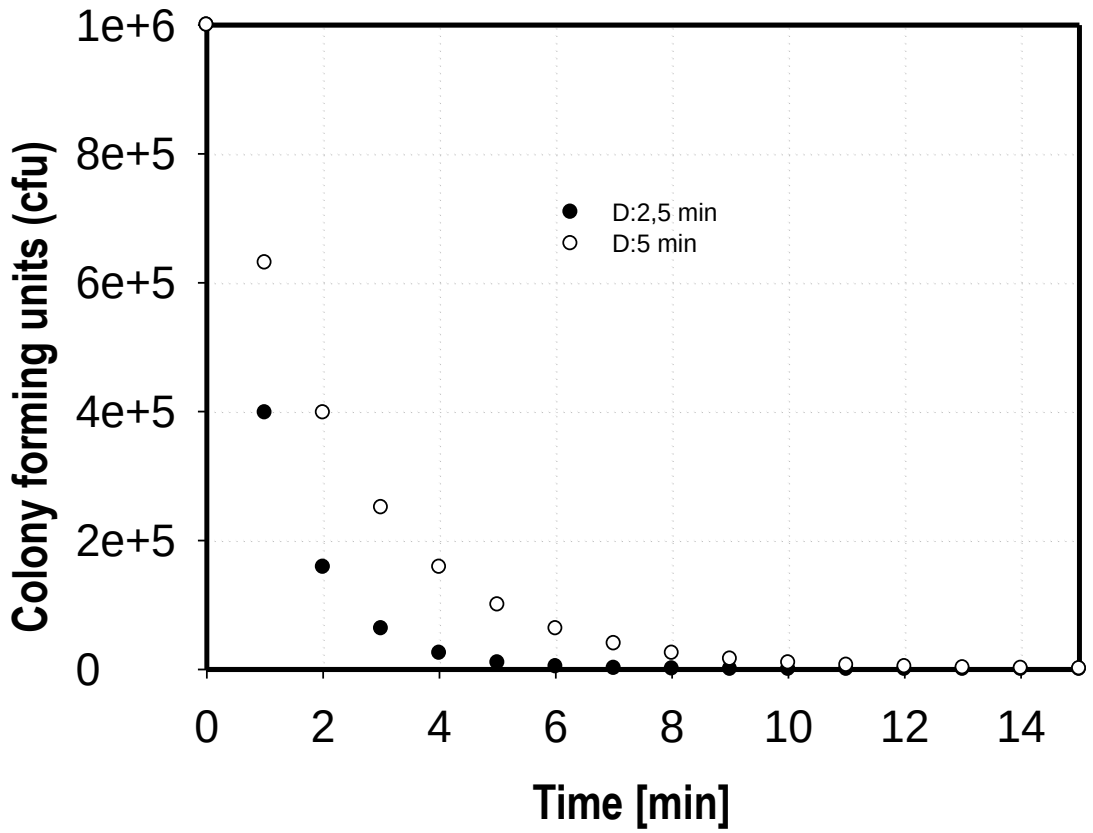


//upload.wikimedia.org/wikipedia/commons/0/02/Dna_repair_base_excersion_de.svg

Regulatory Requirements

- EN ISO 14937 Validation and characterization of sterilization processes in general
- EN 556-1 Defines „sterile“
- EN 556-2 Defines „sterile“ aseptic
- EN ISO 11737-2 Test on steriliy of a product
- EP 2.6.12, USP 35 <61> Bioburden, cultivation conditions
- EP 2.6.13, USP 35 <62> Exclusion of pathogens
- EP 2.6.1 Test on sterility
- EN ISO 11138 Series: Bioindicators
- EN ISO 10993-1 Biocompatibility
- EN ISO 10993-7 EO residuals
- EN ISO 11138-7 Calculation of lethality
- EN ISO 11135 Series EO sterilization
- EN ISO 11137 Series irradiation sterilization
- EN ISO 17665 Series moist heat sterilization
- EN ISO 13408 Aseptic processing

Kill kinetics of microorganisms



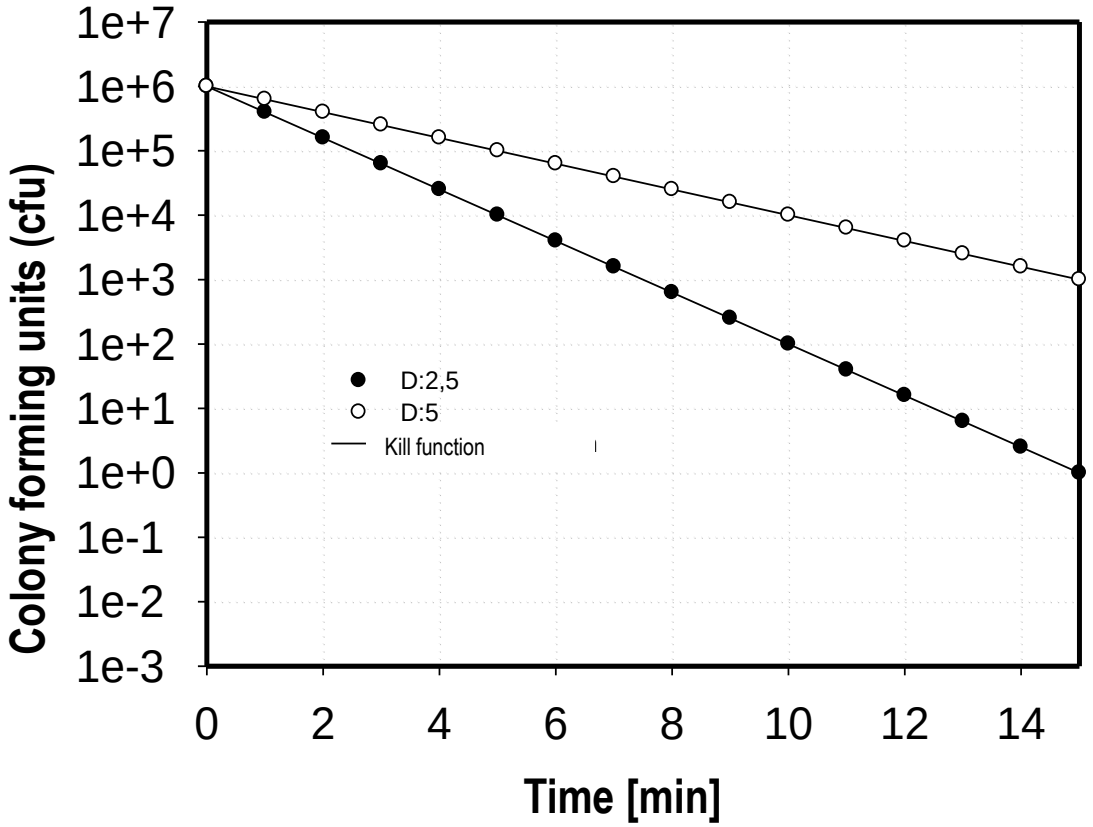
What does „sterile“ mean?

- SAL = 10^{-6} *(Sterility Assurance Level)*
  - 1 viable organism in 1 X 10^{6} products
  - Consequences:
    - Proof of sterility can not be shown– therefore the process has to be validated
    - The process has to be evaluated concerning its influencing factors based on statistics:
      - The requirements can be found in the respective standards for the specific sterilization methods.
    - The complete manufacturing process has to ensure the chosen SAL.

Therefore, only an indirect method is possible:

- *Test of sterility has to be passed (Eu. Ph.2.6.1, EN ISO 11737-2 incl. full validation of the method)*
- *Standard procedure: SAL = 10^{-6} (definition from EN 556-1)*
- *Aseptic processing: SAL = 10^{-3} (definition from EN 556-2)*

Kill kinetics of microorganisms



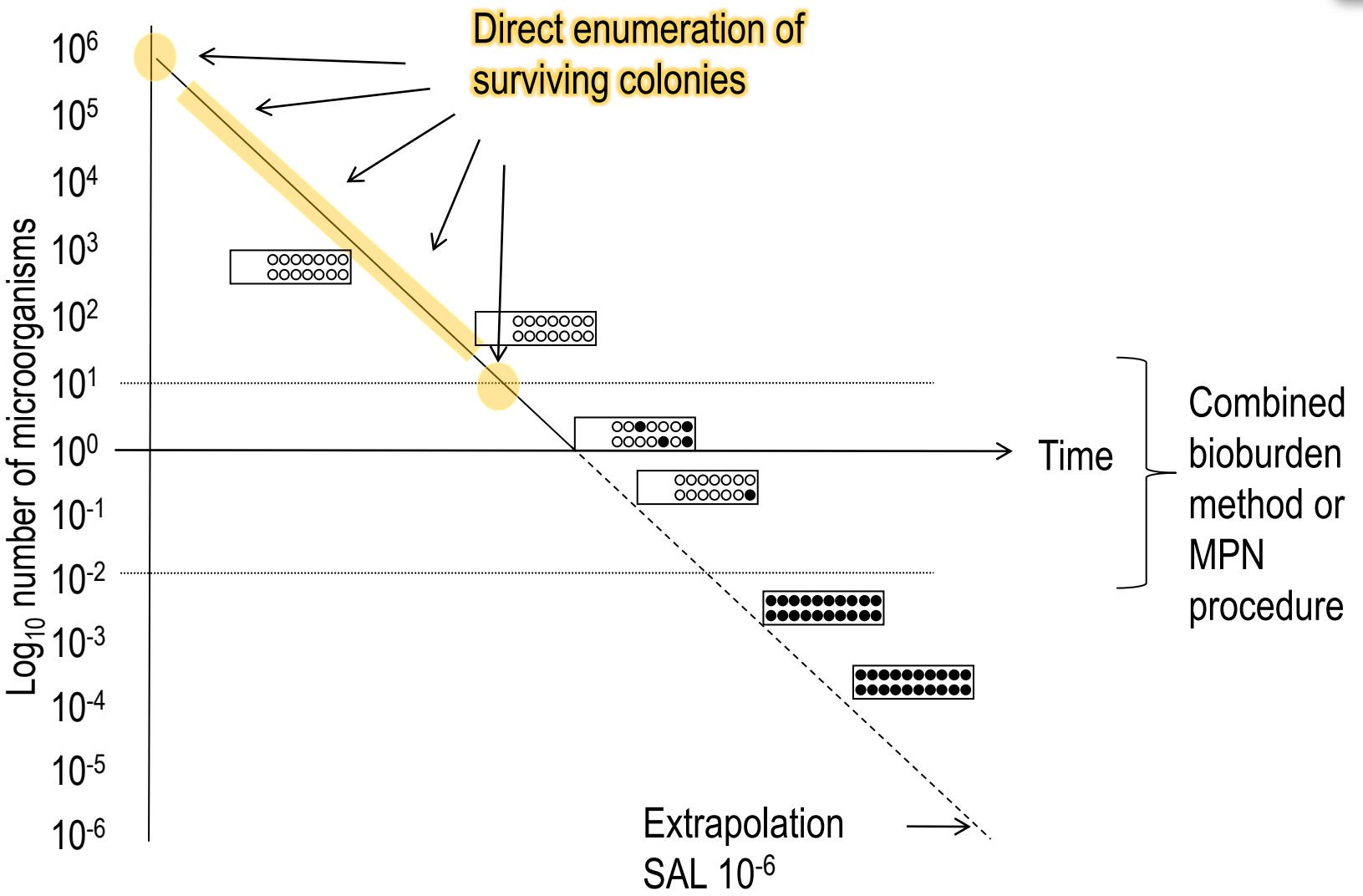
Determination of lethality

Kill kinetics may:

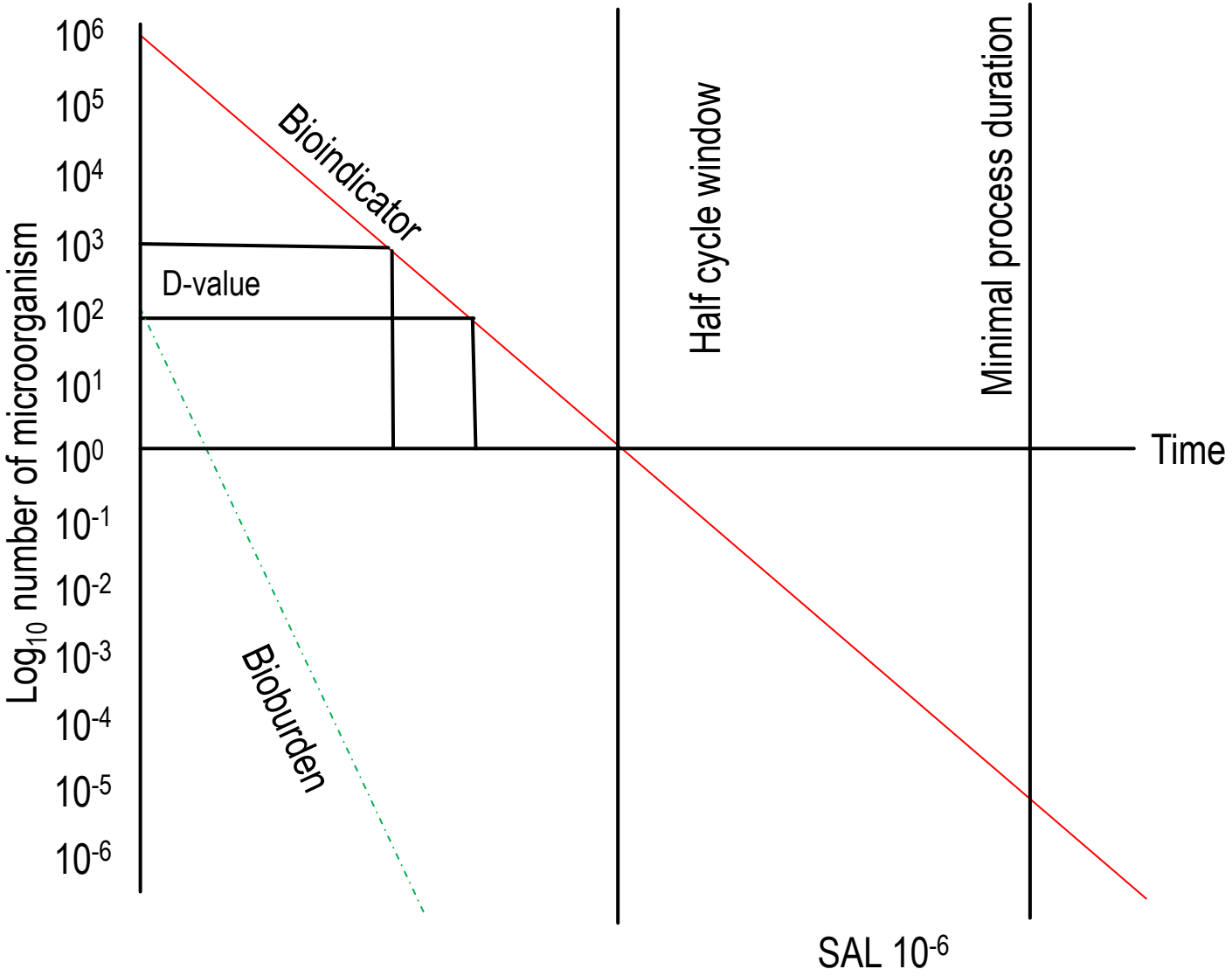
- Be based on the natural bioburden with subsequent test of sterility (ISO 11737-2)
- Be based on a known bioburden and usage of a reference organism - e.g. direct enumeration or (MPN) procedure
- Be based on a conservative overkill approach

ISO 14937 Annex B-D

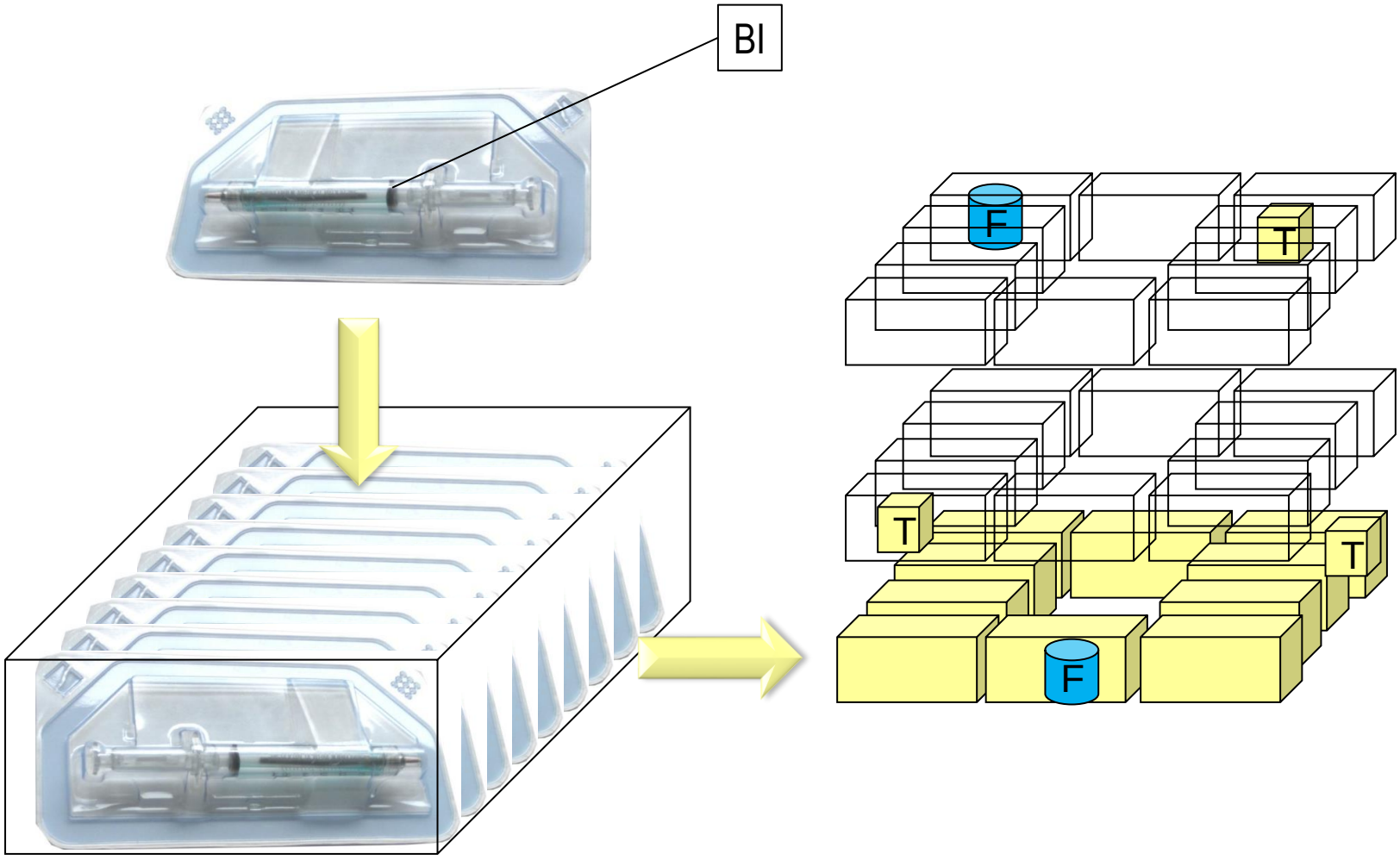
Kill kinetics with reference organism and bioburden



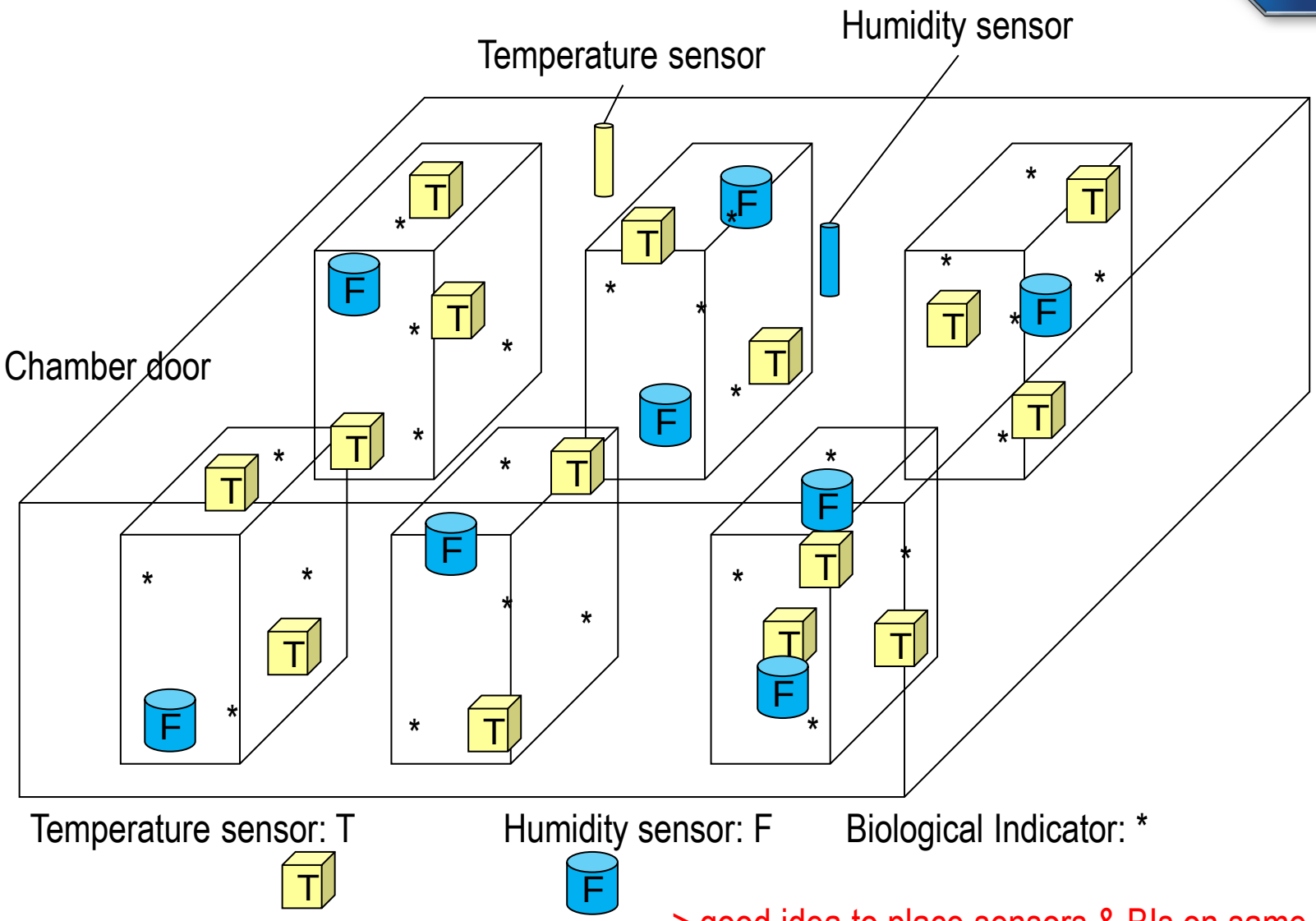
Kill kinetics of microorganisms – Overkill approach



Example EO: Description of pallets – MPQ and PPQ related data



Example EO: arrangement of the sensors



-> good idea to place sensors & BIs on same spot

EN ISO 11135 Annex.

However – Irradiation Sterilization is different in some point

- Why do we not need Bioindicators for irradiation sterilization?

  – Because we rely on the SDR (Standard distribution of resistances)

  – Therefore we have to rely on absolute rigid laboratory results on bioburden and a thorough knowledge of the bioburden of the device:
    - Including its packaging
    - Including bacteria, spores, yeast and molds, anaerobic organism!
    - And resulting Endotoxins

Methods that follow a standard distribution of resistance

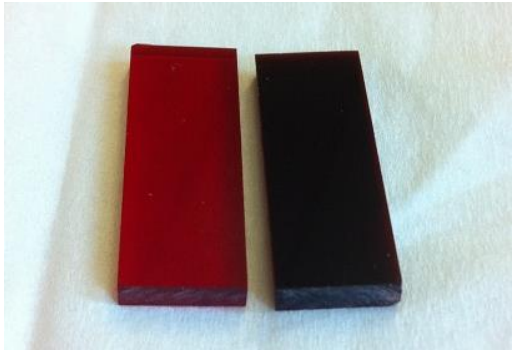
- Method 1, $VD_{max}25$ und $VD_{max}15$:
  - The methods are based on the theory that a population of microorganisms has a standard distribution of resistance against radiation. Consequently:
    - This standard population can be reduced to a desired level by a defined radiation dose (following a mathematical relation)
      - The initial number of microorganisms has to be known for this
    - It can be checked, whether a population on a product follows the mathematical resistance-dose-relation.

- This examination of the dose resistance relation dependent on the initial number of microorganisms is referred to as **verification dose experiment**.
  - Each of the above mentioned methods is following its own function of decimation. The functions are shown in tables in the standard (bioburden data in the tables are to be rounded up)

Dosimetry –measurement of the radiation dose

- Dosimeter
  - An object that can reproducibly measure the applied radiation dose.
  - There are different dosimeters regarding precision and applications
  - Calibration has to be traceable to a National Standard Institute

Red Perspex
(PMMA)
non-irradiated (l)
and irradiated (r)



- Typical dosimeters used in industry:

Type	Range of sensitivity (Gy)	Readout with
Alanine	1 - 10^5	Eletron spin resonance spectrometer
Ceric-sulfate (solution)	10^3- 10^5	UV spectrophotometer
Stained polymethylacrylate (thick film)	10^3- 5 x 10^4	UV-Vis spectrophotometer
Radiochromic film	1 - 10^5	UV-Vis spectrophotometer

EN ISO 11137-1 4.3.4

Effects and drawbacks on product by sterilization method

- Irradiation
  - Material change due to cross linking of polymers – tension cracks, increase of TOC
  - Artificial ageing of sensible materials – e.g. packaging, discoloring
  - Incompatibility of most electrical products
  - Shielding effects with metallic objects
  - Load is a fixed parameter – any load/product change needs an own dose mapping
    - Prone to wrong decisions in relation to family grouping
    - Prone to wrong decisions in relation to bioburden handling and method development

- EO
  - EO residuals and related long aeration times – material dependent (PVC, PUR...)
    - Special handling of pediatric devices
  - Reaction of EO with coatings and drugs
  - Packaging has a direct influence on the sterilization outcome
  - some organism are hard to kill (fungi, spores)
  - Product design has to allow easy entering of the moisture and gas
  - Batteries in products need special sterilization cycles

Typical documentation for sterilization processes

- Minimal documentation:
  - Validation protocol (incl. all relevant process parameters + revalidation criteria)
  - Validation report (IQ,OQ,**PQ**) + Revalidation data)
  - Certificates of the cooperating sites (incl. calibration, microbiological laboratory, Sterilization supplier)
  - Results of the microbiological laboratory (bioindicators, bioburden trending throughout the year, treatment parameter of bioindicators + positioning in the load and in the product, biological testing data on residues)
  - Raw data of the physical performance qualification (lot traceability!)
  - Raw data of microbiological performance qualification (lot traceability!)
  - Routine release criteria (based on validation output) and evidences
  - Maintenance requirements and related data
  - Training evidence of operators

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Thank you for your attention!

Further information to Technical Dokumentation on sterilization you may find here:

[Biological Safety Checklists - Medical Devices | TÜV SÜD (tuvsud.com)]