Introduction to current sterilization methods

Hydrogen peroxide decontamination

Lucia Maita, Technical Customer Support, Fedegari Group

Agenda

Introduction
Decontamination, Gas sterilization & Vapour phase sterilization

Hydrogen peroxide
Chemical properties & Sporicidal Mechanism

Decontamination technologies
Fedegari Isolation technology

Development & Validation of a decontamination process
Normative references & practical case studies

Decontamination

The removal of microorganisms by **disinfection** or **sterilization**.

Disinfectant—A chemical or physical agent that destroys or removes vegetative forms of harmful microorganisms when applied to a surface.

Sterilant—An agent that destroys all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores. Sterilants are liquid or vapor-phase agents.

USP43 ⟨1072⟩ Disinfectant and Antiseptics

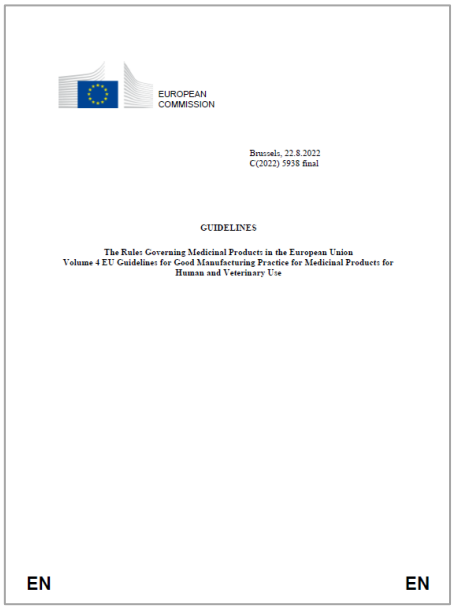
Decontamination

…“decontaminated” refers to an **item** or **surface** that has been subjected to a **process that eliminates viable bioburden**.

USP43 ⟨1208⟩ Sterility Testing – Validation of Isolator System

Decontamination

The overall process of **removal or reduction of any contaminants** (chemical, waste, residue or microorganisms) from an area, object, or person. The method of decontamination used **(e.g. cleaning, disinfection, sterilisation)** should be chosen and validated to achieve a level of cleanliness appropriate to the intended use of the item decontaminated. See also Bio-decontamination.


EUROPEAN COMMISSION

Brussels, 22.8.2022
C(2022) 5938 final

GUIDELINES

The Rule: Governing Medicinal Products in the European Union
Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use

EN EN

Bio - Decontamination: A process that eliminates viable bioburden via use of sporicidal chemical agents.

EudraLex Vol. 4 - Annex 1 – Glossary

Gas sterilization (vapour phase sterilization)

Gas sterilization of surfaces may be used for the sterilization of *primary packaging materials*, *equipment* and some *pharmaceuticals*.

European Pharmacopoeia 10.0 – 5.1.1 Methods of preparation of sterile products- Gas sterilisation (vapour phase sterilization)

Sterilizing agent: *physical or chemical entity or combination of entities, having sufficient microsporicidal activity to achieve sterility under specified conditions.*

ISO 11139:2018, 3.288

Sporicidal Vapor Phase Decontamination

The destruction or inactivation of microbial spores using a **vapor** *or* **gaseous agent**.

PDA TR. No. 51, Glossary of Terms

Sterilization

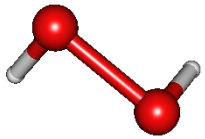
A process used to render a product, environment or pieces of equipment free from viable microorganisms with a specific probability.

PDA TR. No. 51, Glossary of Terms

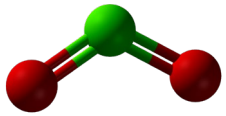
Vapor Phase Sterilization

Sterilization can be accomplished using *sporicidal agents suspended in air* (i.e., vapor). Sterilizing agents that operate in this fashion include *hydrogen peroxide* (H_2O_2), *peracetic acid* (CH_3CO_3CH), *formaldehyde* (CH_2O), and *glutaraldehyde* [$CH_2(CH_2CHO)_2$] in aqueous solution.

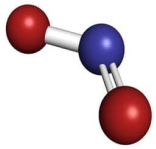
USP43 ⟨1229 .11⟩ Vapor phase sterilization



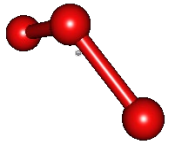
Hydrogen Peroxide
H_2O_2



Chlorine dioxide
ClO_2



Nitrogen dioxide
NO_2



Ozone
O_3

General Classification of Antiseptics, Disinfectants, and Sporicidal Agents

Chemical Entity	Classification	Example
Aldehydes	Sporicidal agent	2% Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70% Isopropyl alcohol, 70% alcohol
Chlorine and sodium hypochlorite	Sporicidal agent	0.5% Sodium hypochlorite
Phenolics	General purpose disinfectant	500 µg per g Chlorocresol, 500 µg per g chloroxylenol
Ozone	Sporicidal agent	8% Gas by weight
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g H_2O_2 vapor, 10%–25% solution, 3% solution
Substituted diguanides	Antiseptic agent	0.5% Chlorhexidine gluconate
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2% Peracetic acid, 1 µg per g peracetic acid
Ethylene oxide	Vapor-phase sterilant	600 µg per g Ethylene oxide
Quaternary ammonium compounds	General purpose disinfectant, antiseptic	Concentration dependent on application, Benzalkonium chloride
β-Propiolactone	Sporicidal agent	100 µg per g β-Propiolactone

USP43 ⟨1072⟩ Disinfectants and antiseptics

The Resistance of Some Clinically Important Microorganisms to Chemical Disinfectants

(Listed in Order of Decreasing Resistance)

Type of Microorganisms	Examples
Bacterial spores	*Bacillus subtilis* and *Clostridium sporogenes*
Mycobacteria	*Mycobacterium tuberculosis*
Nonlipid-coated viruses	Poliovirus and rhinovirus
Fungal spores and vegetative molds and yeast	*Trichophyton*, *Cryptococcus*, and *Candida* spp.
Vegetative bacteria	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella* spp.
Lipid-coated viruses	Herpes simplex virus, hepatitis B virus, and human immunodeficiency virus

USP43 ⟨1072⟩ Disinfectant and Antiseptics

Sporicidal process

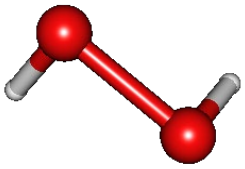
A gaseous, vapour or liquid treatment applied to **surfaces**, using an agent that is recognised as capable of killing bacterial and fungal spores.

….. The process is applied to *internal surfaces of the isolator* and **external surfaces of materials** *inside the isolator*, when conventional sterilization methods are not required.

The application of a sporicidal process to isolators is not considered to be a sterilization process in the same way as, for example, a sealed container subjected to a validated dry heat, moist heat or irradiation process.

PIC/S - Recommendation used for aseptic processing and sterility testing, Glossary

Hydrogen Peroxide – H_2O_2



PubChem

Hydrogen Peroxide is a peroxide and **oxidizing agent** with **disinfectant**, **antiviral** and **anti-bacterial** activities. Upon rinsing and gargling or topical application, hydrogen peroxide exerts its **oxidizing activity** and produces *free radicals which leads to oxidative damage to proteins and membrane lipids*. This may inactivate and destroy pathogens and may prevent spreading of infection.

H_2O_2 Decontamination: when?

Heat-sensitive materials (including electronic devices) that should be transferred between classified areas (class C,D → class A, B) in order to minimize the risk of contamination

H_2O_2 Decontamination: where?

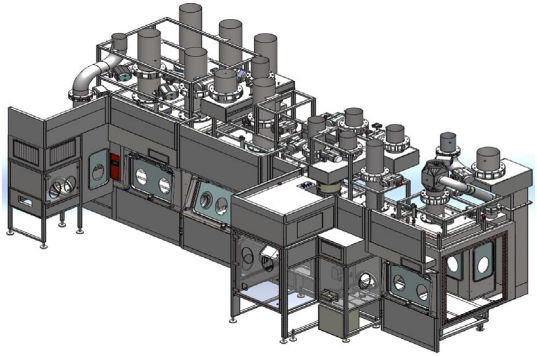
Fedegari Isolator technology

FCDV

Fedegari Cabinet Decontamination Vapor

FCTS

Fedegari Sterility Test Isolator



Customizable solutions

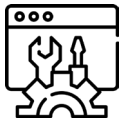
Flexible Filling Line Isolator

An effective H_2O_2 decontamination: how?

The biocide should reach **all the surfaces** that should be decontaminated at a **specific concentration** for a pre-determined **amount of time**.

This means that the biocide must have:

- Good and complete **distribution**
- Sufficient contact **time** at a specified **concentration**

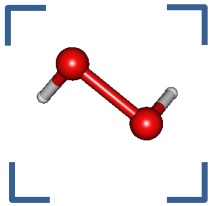


Items exposed to the process should have their surfaces exposed to the greatest extent possible

… There are several effective approaches to hydrogen peroxide (H_2O_2) injection, including **continuous**, **intermittent**, or **all at once**. Some of the systems utilize an **evacuation or drying step** prior to introduction of the hydrogen peroxide (H_2O_2) to allow for increased concentration without excess condensation. Alternatively, hydrogen peroxide (H_2O_2) can be introduced as a **liquid, followed by target heating**. Following the exposure period, the chamber or target is **aerated** to an acceptable level for further processing of materials and/or personnel exposure (whichever is lowest) prior to opening and removing the sterilized article.

USP43 ⟨1229.11⟩ – Vapor Phase sterilization

Typical process conditions



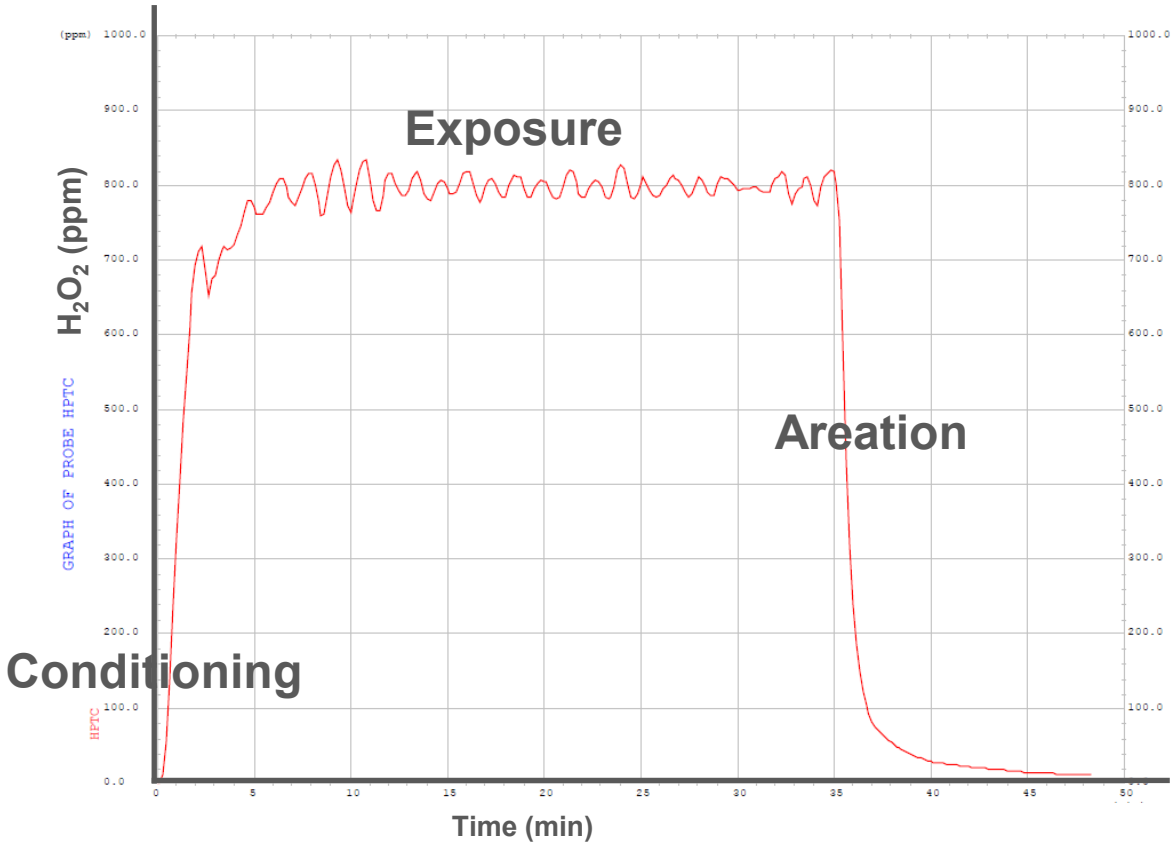
700 –1300 ppm H_2O_2 vapor during decontamination

≤ 5 – 90 % Humidity

≤ 25°C Temperature during the process

Total cycle time: based on the product type

Decontamination Cycle



An effective H_2O_2 decontamination: how?

A combination of **physical** and **biological methods** is used to determine the **optimum decontamination conditions**.

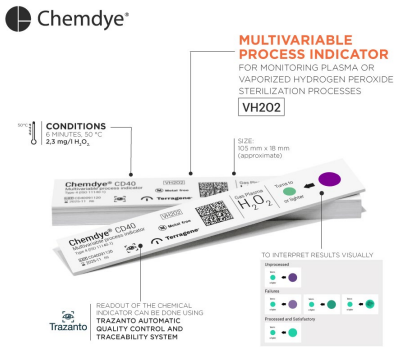
- Biocide concentration
- Process temperature
- Relative humidity
- Load configuration
- Exposure time

- Chemical Indicator
- Biological Indicator

H_2O_2 distribution

… **chemical indicators** can provide a limited indication of sterilant distribution.

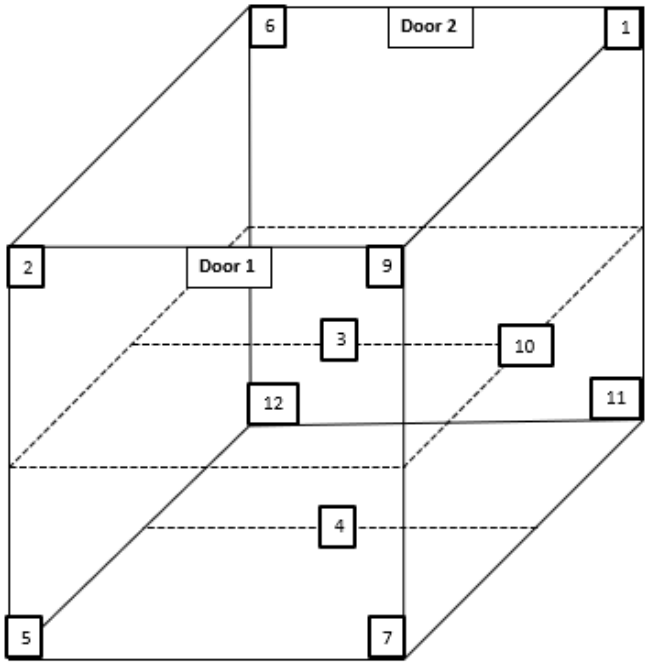
USP 43 ⟨1229.11⟩ Vapor phase sterilization



Chemical Indicator: Test system that reveals change in one or more pre-defined process variables based on a chemical or physical change resulting from exposure to a process

UNI EN ISO 17665, Terms and Definition 3.6

H_2O_2 distribution: Empty chamber mapping



Exposed

Not Exposed

H_2O_2 distribution: BIs & H_2O_2 Process Validation

Biological indicators are required to **demonstrate the effectiveness of the process** they play a crucial role during the cycle development and validation.

USP 43 ⟨1229.5⟩ and PIC/S Recommendation clause 9.4.13

Biological Indicator: It is a well-characterized preparation of a specific microorganism that has know resistance to a specific sterilization process.

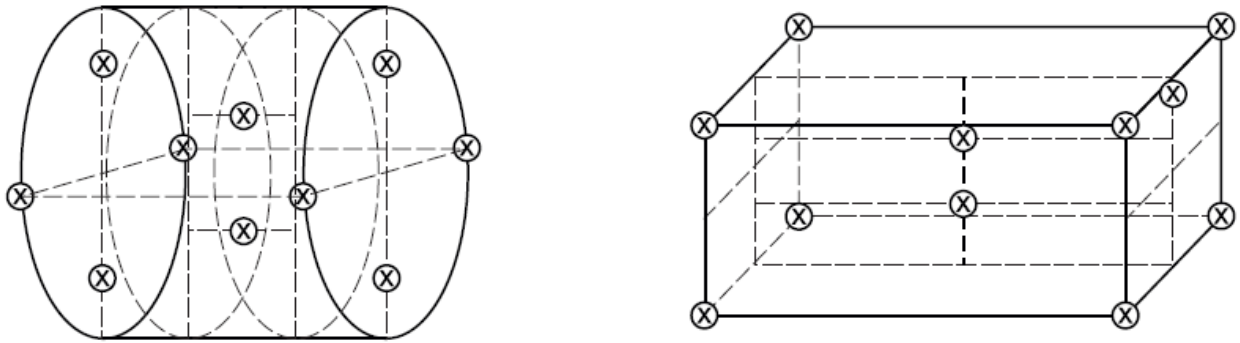
USP 43 ⟨1229.5⟩ Biological Indicators For Sterilization

The use of **multiple BIs** at each test location is recommended to more adequately support the process lethality.

USP 43 ⟨1229.11⟩ Vapor phase sterilization

H_2O_2 distribution: BIs & H_2O_2 Process Validation

The BIs should be evenly **distributed throughout the camber and the load**



Key

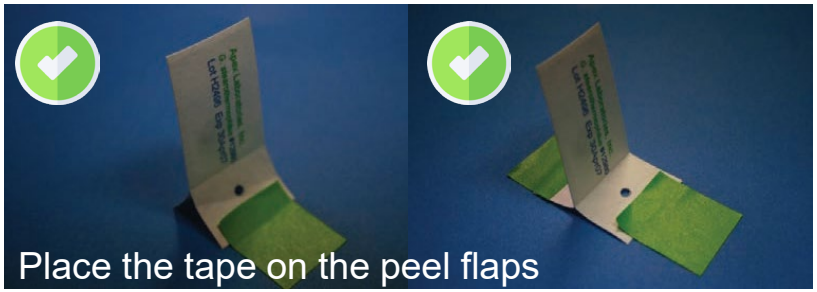
X locations for BIs, PCDs or temperature sensors

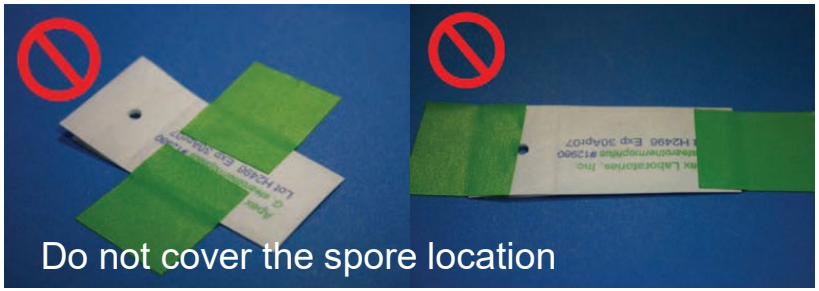
NOTE The diagram shows examples for locations in typical chamber usable space. Different chamber sizes can require more or fewer locations however a similar distribution pattern can be used.

ISO 22442 – Annex K (informative) Recommended validation test procedures

BI placement & handling

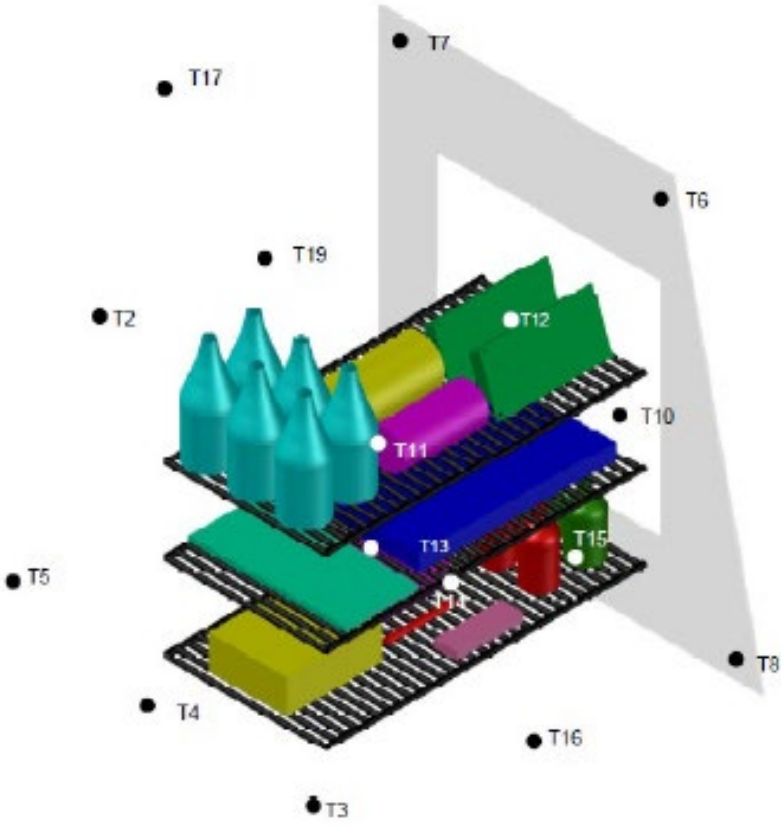
Proper BI Placement During VHP Decontamination Cycles - Kurt McCauley Spore News MesaLabs Volume 9, No. 5





BIs & CIs Layout

T1 Left corner of the sealing front side
T2 Left corner of the sealing backside
T3 Left corner of the bottom front side
T4 Left corner of the bottom backside
T5 Middle of the left wall
T6 Right corner of the sealing front side
T7 Right corner of the sealing backside
T8 Right corner of the bottom front side
T9 Right corner of the bottom backside
T10 Middle of the right wall
T11 Between the bottle and Petri package - top shelf
T12 Between the canisters - top shelf
T13 Middle of the middle shelf
T14 Middle of the bottom shelf
T15 Between the bottle of the bottom shelf, the left side
T16 Middle of the bench
T17 Middle of the ceiling
T18 Middle of the front wall (transfer unit door)
T19 Middle of the back wall

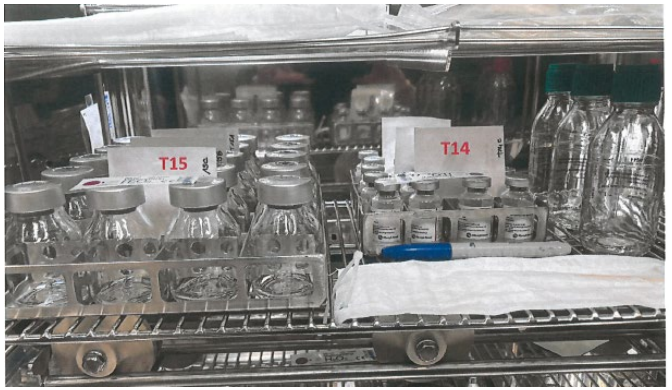


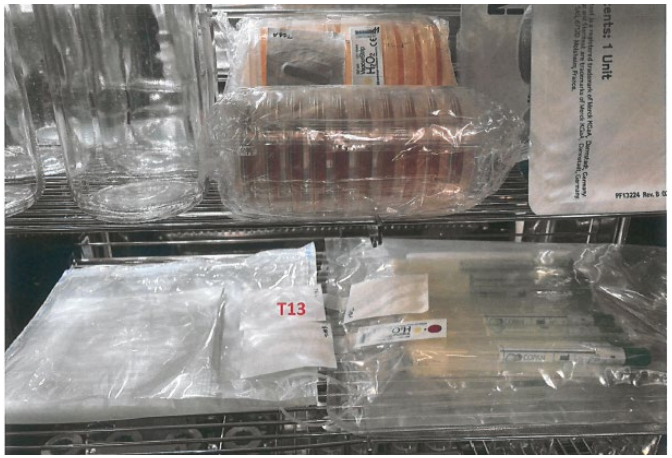
BIs & CIs Mapping

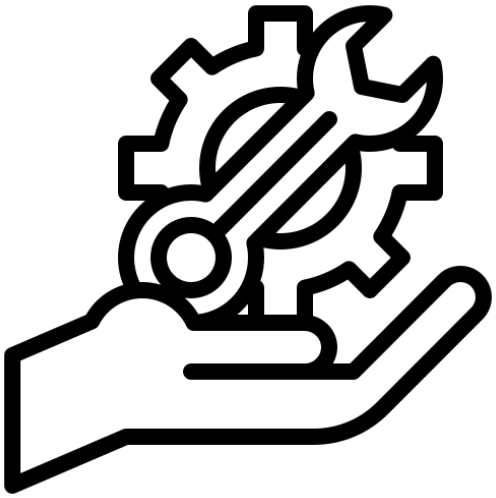
T1 Left corner of the sealing front side
T2 Left corner of the sealing backside
T3 Left corner of the bottom front side
T4 Left corner of the bottom backside
T5 Middle of the left wall
T6 Right corner of the sealing front side
T7 Right corner of the sealing backside
T8 Right corner of the bottom front side
T9 Right corner of the bottom backside
T10 Middle of the right wall
T11 Between the bottle and Petri package - top shelf
T12 Between the canisters - top shelf
T13 Middle of the middle shelf
T14 Middle of the bottom shelf
T15 Between the bottle of the bottom shelf, left side
T16 Middle of the bench
T17 Middle of the ceiling
T18 Middle of the front wall (transfer unit door)
T19 Middle of the back wall

BIs & CIs Mapping

3 BIs and 1 CI at each chosen position

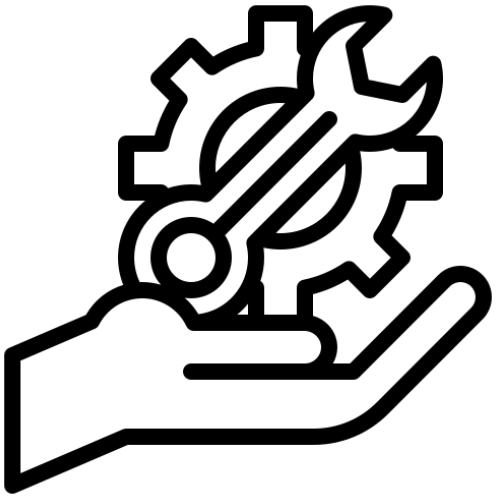




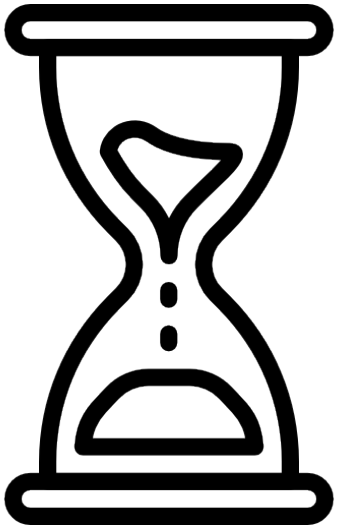


PRACTICAL CASE STUDY

Simulation of a Passbox reduced load qualification



1. **Chamber loading** according to the layout
2. **CIs placement**
3. **BIs placement**



KEEP CALM

and wait for ....

...1 ppm!

Thank you for your attention