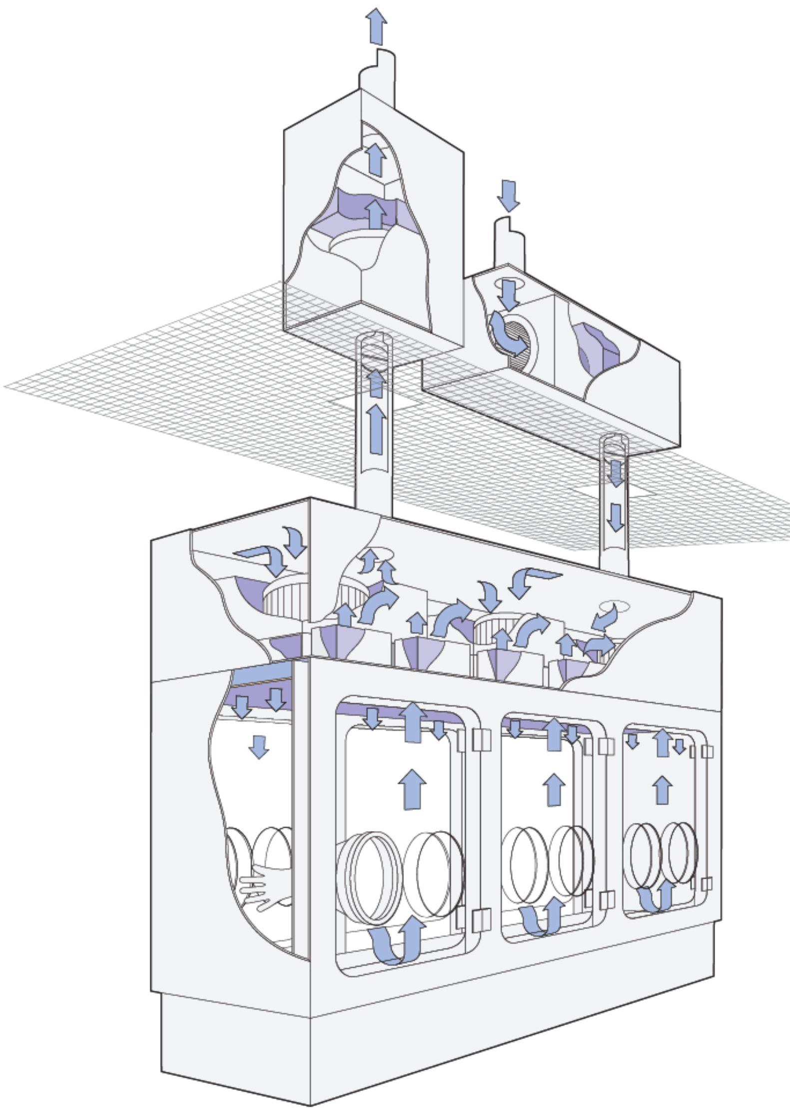
Bio-decontamination with Hydrogen Peroxide (H_2O_2): Fundamentals

Parenteral Drug Association

Isolator technology

- Separation of the process and operators
- Aseptic processing ~ handling of a product while preventing its (microbial) contamination

- Key Functions
  - Maintenance of Aseptic state
    - HEPA filtration
    - Unidirectional airflow
    - Differential pressure (gradient)
    - Transfer systems
    - Physical separation (gloves)
  - Establishment of aseptic state
    - (Cleaning / Disinfection)
    - Bio-decontamination
    - (Sterilization)



Bio-decontamination

“A process that eliminates viable bioburden via use of sporicidal chemical agents”
glossary of EU GMP Annex 1

Key applications

- Bioburden management: room bio-decontamination, material transfer airlocks/hatches
- In preparation of an isolator/enclosure for aseptic processing (production)

Expectations on bio-decontamination

- Automated (and integrated)
- Quantifiable and Parametrized
- Reproducible / Robust
- Validated

Requirements are ever increasing propelled by (bio)pharmaceutical industry evolution

- Fast cycles (productivity, cold chain, stability)
- Lower H_2O_2 residues (no impact on the product or aseptic processes)
- Flexibility / adaptability (various load patterns)
- Sustainability (small footprint, air-reuse)

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

Annex 1

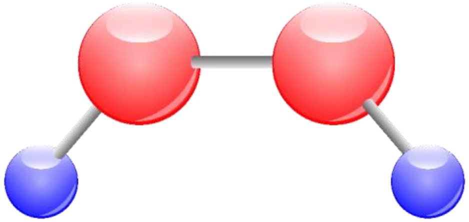
Manufacture of Sterile Medicinal Products

4.22
i. For isolators

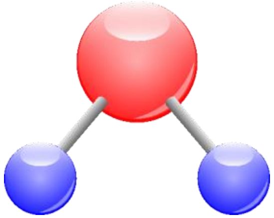
The bio-decontamination process of the interior should be automated, validated and controlled within defined cycle parameters and should include a sporicidal agent in a suitable form (e.g. gaseous or vaporized form). Gloves should be appropriately extended with fingers separated to ensure contact with the agent. Methods used (cleaning and sporicidal bio-decontamination) should render the interior surfaces and critical zone of the isolator free from viable microorganisms.

Hydrogen peroxide (H_2O_2)

- Why do we use H_2O_2 ?
  - Broad non-specific activity against microorganisms
  - Low toxicity, safe to use
  - Active at low temperatures and ambient pressure
  - Good material compatibility
  - Acceptable storage stability
  - Environmentally green solution
- Why vapor form ?
  - Complex, yet highly effective
  - Vapor may be efficiently distributed over the enclosure
  - It allows automated “No touch” process that can be validated
  - Established technology
    -> over 25 years of successful history



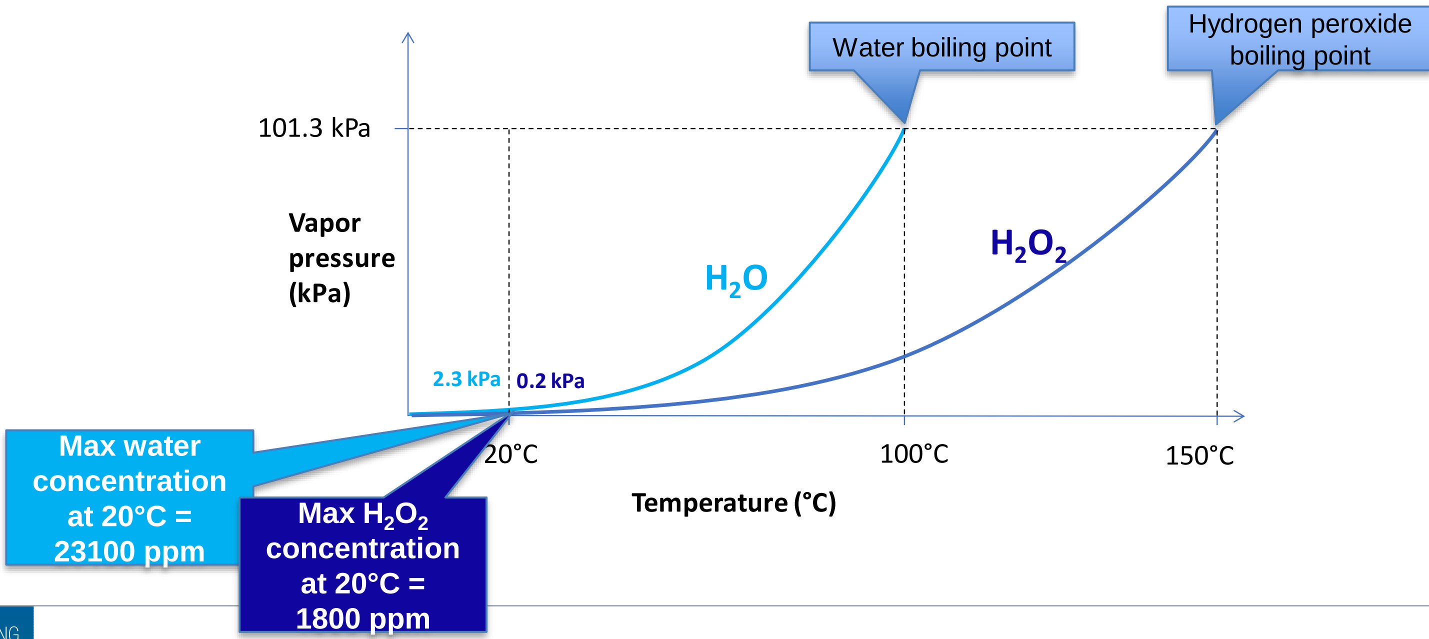
Hydrogen peroxide
BP: 150°C / 302°F



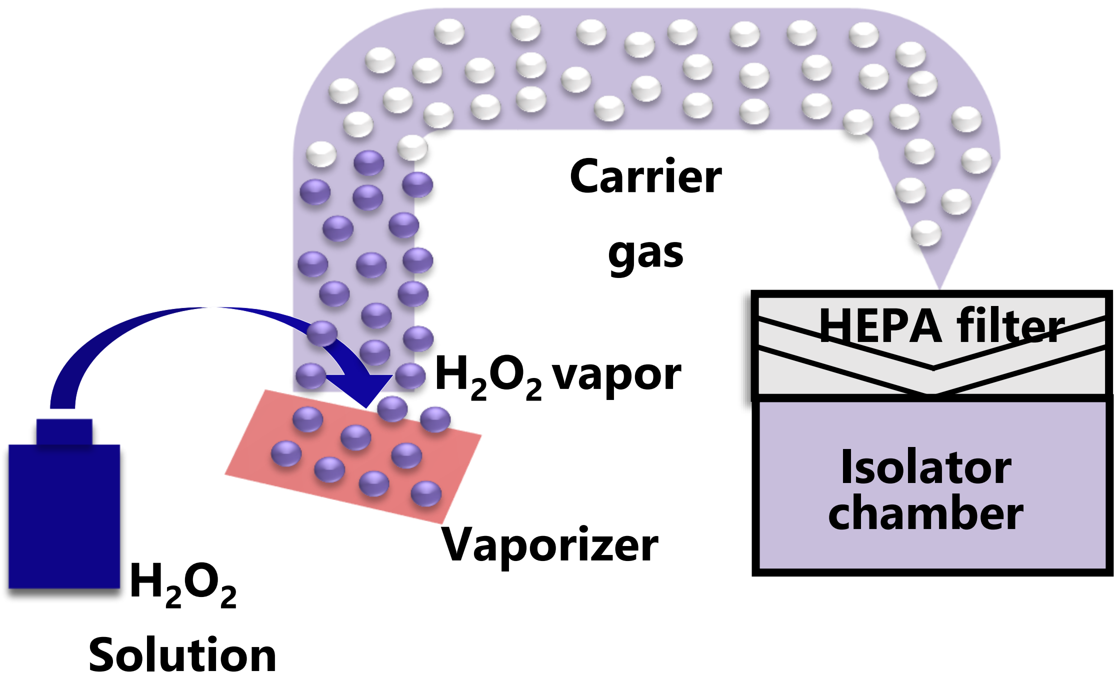
Water
BP: 100°C / 212 °F

Vapor

- Vapor refers to molecules in a gas phase of a substance that at given temperature exists as a liquid (or a solid)
- Each substance has a limit (maximal) vapor concentration depending on the temperature "Saturation vapor pressure"
- H_2O_2 is less volatile than water (approx. 10x) -> evaporated H_2O_2 condenses preferably



Bio-decontamination basic principle



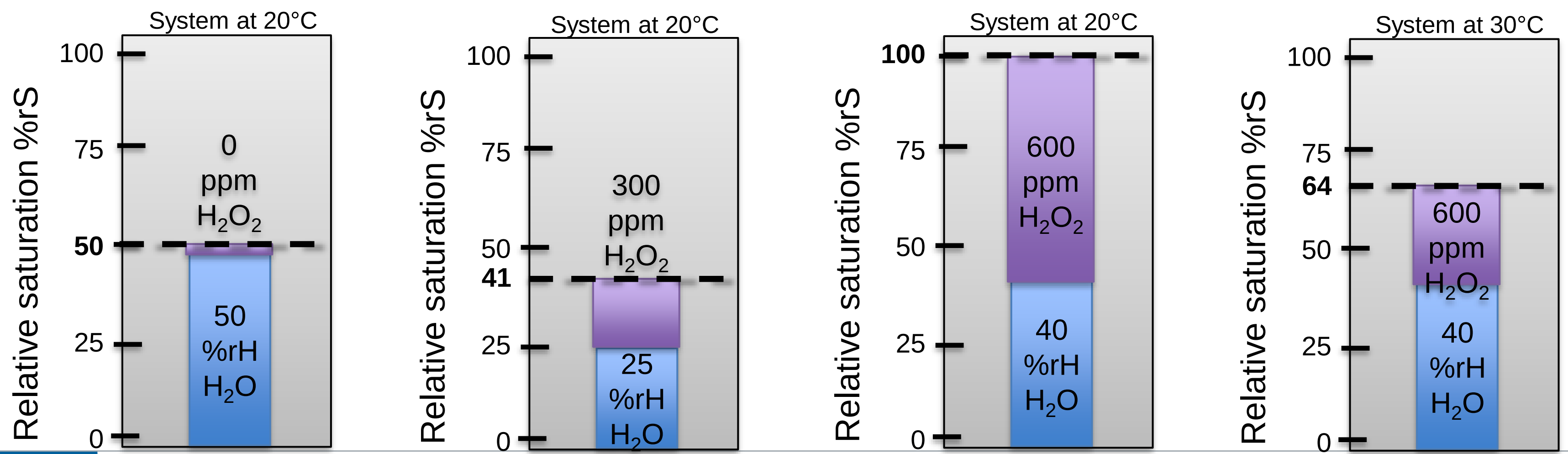
Vaporization → Distribution → Deposition → Removal

Bio-decontamination agents

- Following species act during bio-decontamination:
  - Air molecules - present as gas
  - Water (H_2O) molecules - present as gas (i.e. vapor) or as liquid (i.e. droplets/condensate)
  - H_2O_2 molecules - present as gas (i.e. vapor) or as liquid (i.e. droplets/condensate)
- H_2O_2
  - The “active” agent - responsible for the bio-decontamination effect
  - Its distribution (homogeneity and concentration) and its form (vapor/liquid) are important
  - It is influenced by humidity and temperature fluctuations as well as by materials in contact
  - Gradually decomposes to water and oxygen
- Water
  - The “influencing” agent – it impacts H_2O_2 vapor/liquid equilibrium (H_2O_2 condensation)
  - It swells proteins and influences oxidative radical reactions
- Air
  - The “inert”
  - It may be used to accelerate H_2O_2 distribution by translational movements (active mixing)
  - It slows down the diffusion rate of H_2O_2/water molecules

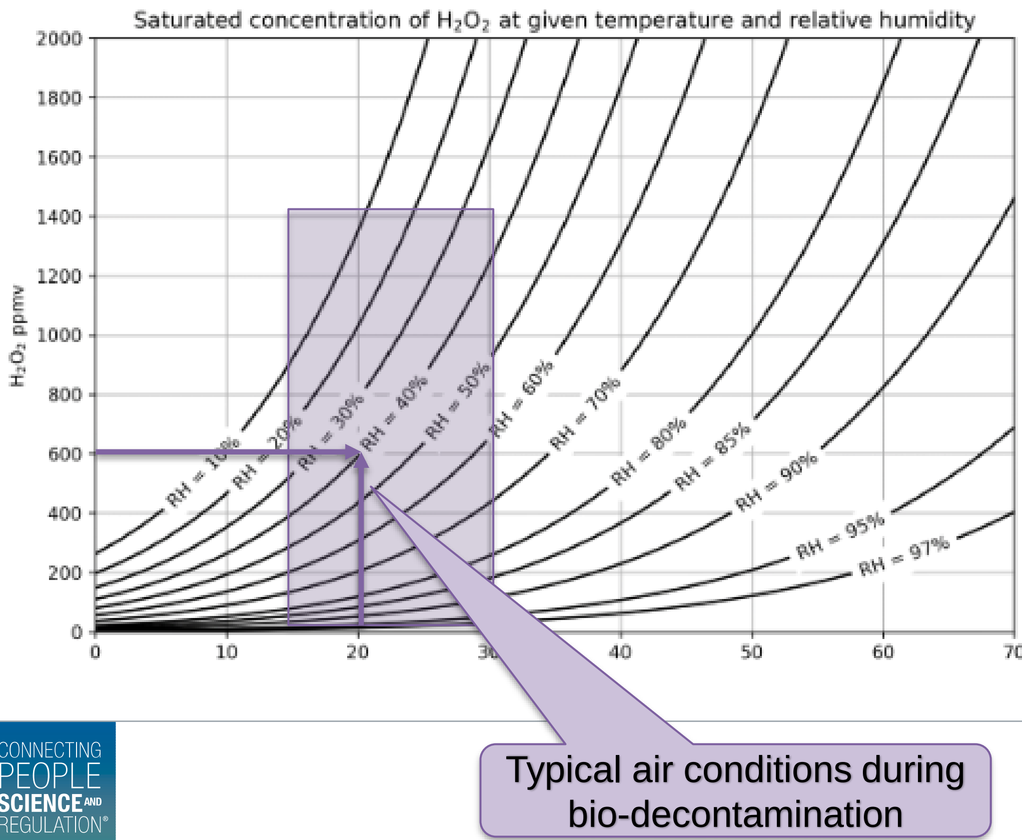
Humidity and Saturation

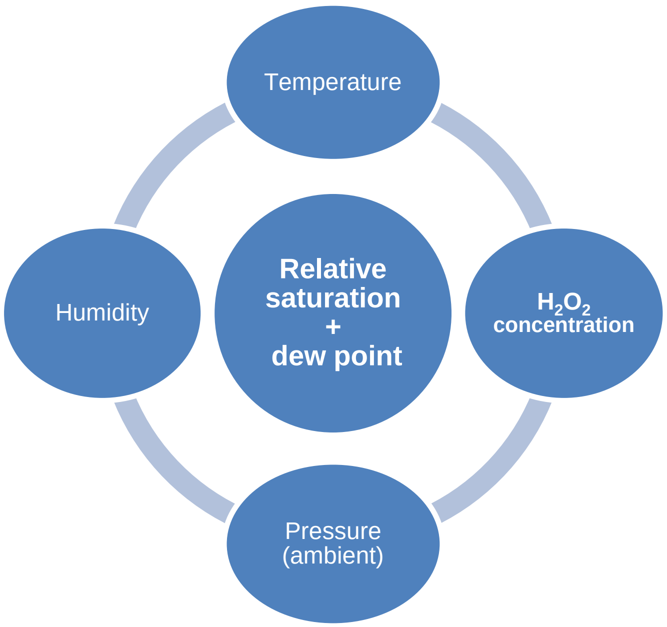
- Relative Humidity (%rH) represents the amount of water vapor in the air
  - Not “directly” related to killing, but of importance for its effect on relative Saturation
- Relative Saturation (%rS) represents the combined amount of water and H_2O_2 vapor in air
  - Relative saturation is used to express the remaining vapor capacity of air
  - In other words, it expressed the “willingness” of H_2O_2-water vapor to condense
- Lower relative humidity ↓ -> higher maximal H_2O_2 vapor concentration ↑
- Higher temperature ↑ -> higher maximal H_2O_2 vapor concentration ↑



Key bio-decontamination parameters

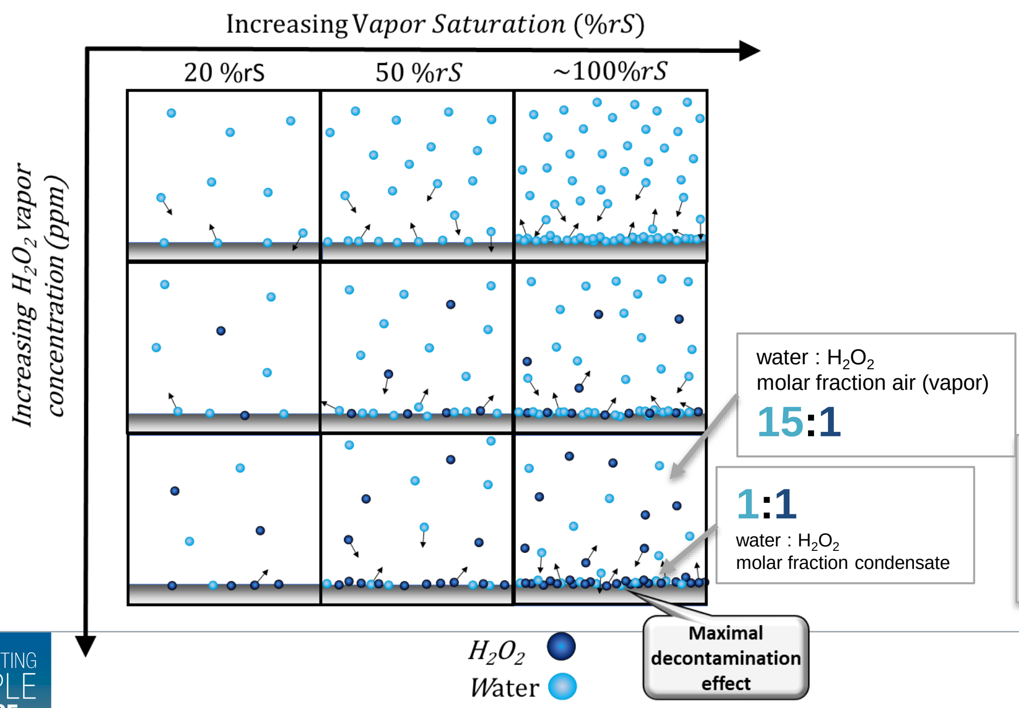
- Key parameters: CONTACT TIME, H_2O_2 vapor concentration and relative saturation
- Microbial inactivation rate increases (=> better bio-decontamination effect) with
  - Longer contact time, higher H_2O_2 vapor concentration, higher relative saturation





H_2O_2 deposition adsorption + (micro)condensation

- Deposition appears on all surfaces in contact with hydrogen peroxide/water vapor
- The deposited amount increases with
  - Increasing relative saturation
  - Increasing H_2O_2 concentration
  - Decreasing surface temperature



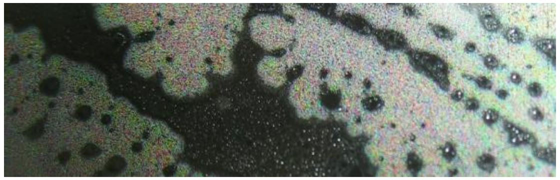
Adsorption (invisible)

↓

Micro-condensation (sub-visible)

↓

Visible condensation



Example on – vapor-liquid equilibrium
20°C, 600ppm H_2O_2, 40%rH (9000ppm water)
-> deposition of 60%wt H_2O_2

H_2O_2 distribution in-homogeneities

“H_2O_2 bio-decontamination effect is never perfectly homogeneous, and it is not required“

Sources of localized H_2O_2 effect variations:
-> H_2O_2 vapor source/injector positioning, means of H_2O_2 distribution, airflow pattern
-> Isolator shape, equipment and loading pattern/ configuration
-> Material properties and its cleanliness
-> Localized variations of temperature (and humidity)

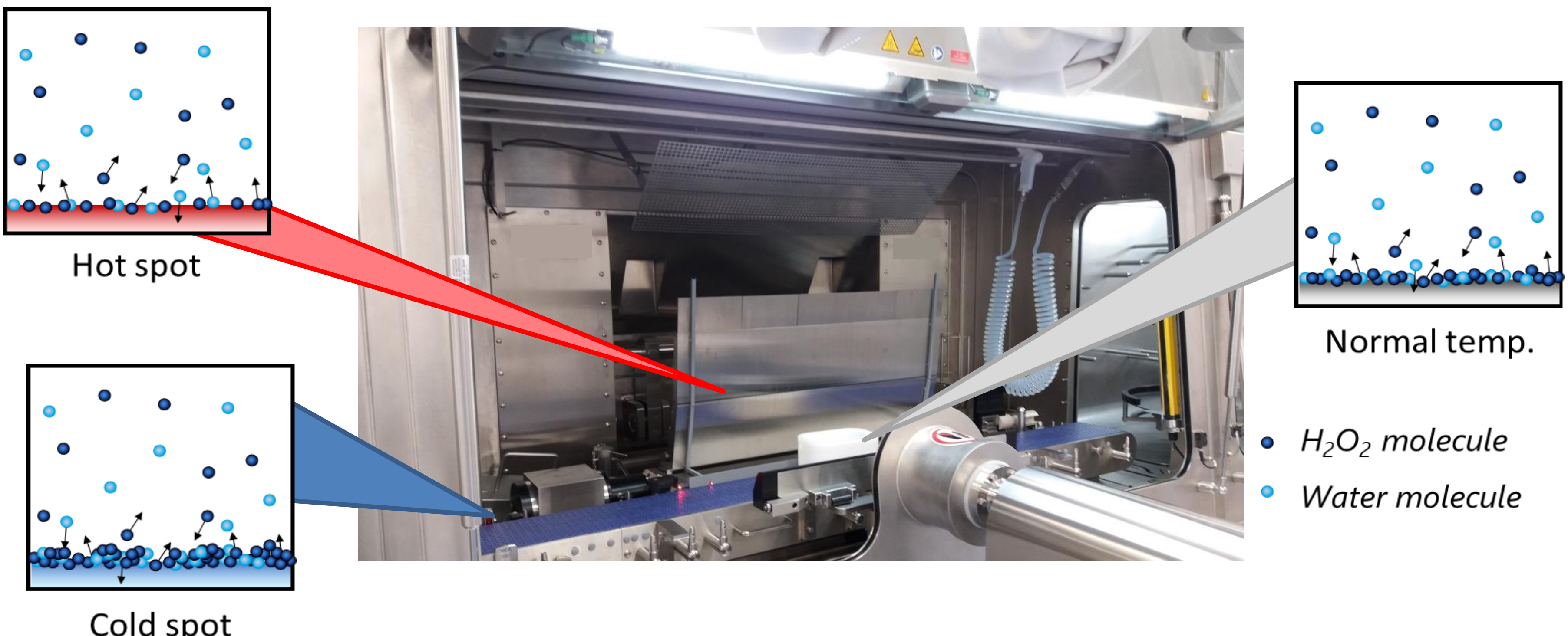
=> Robust technology and proven validation strategy => Successful application

Empty chamber

Loaded chamber

Effect of temperature locally

- Deposition of H_2O_2 on a surface decreases with increasing surface temperature
- Importance of temperature mapping for cycle development



Simple principal of bio-decontamination

Vaporization

Distribution

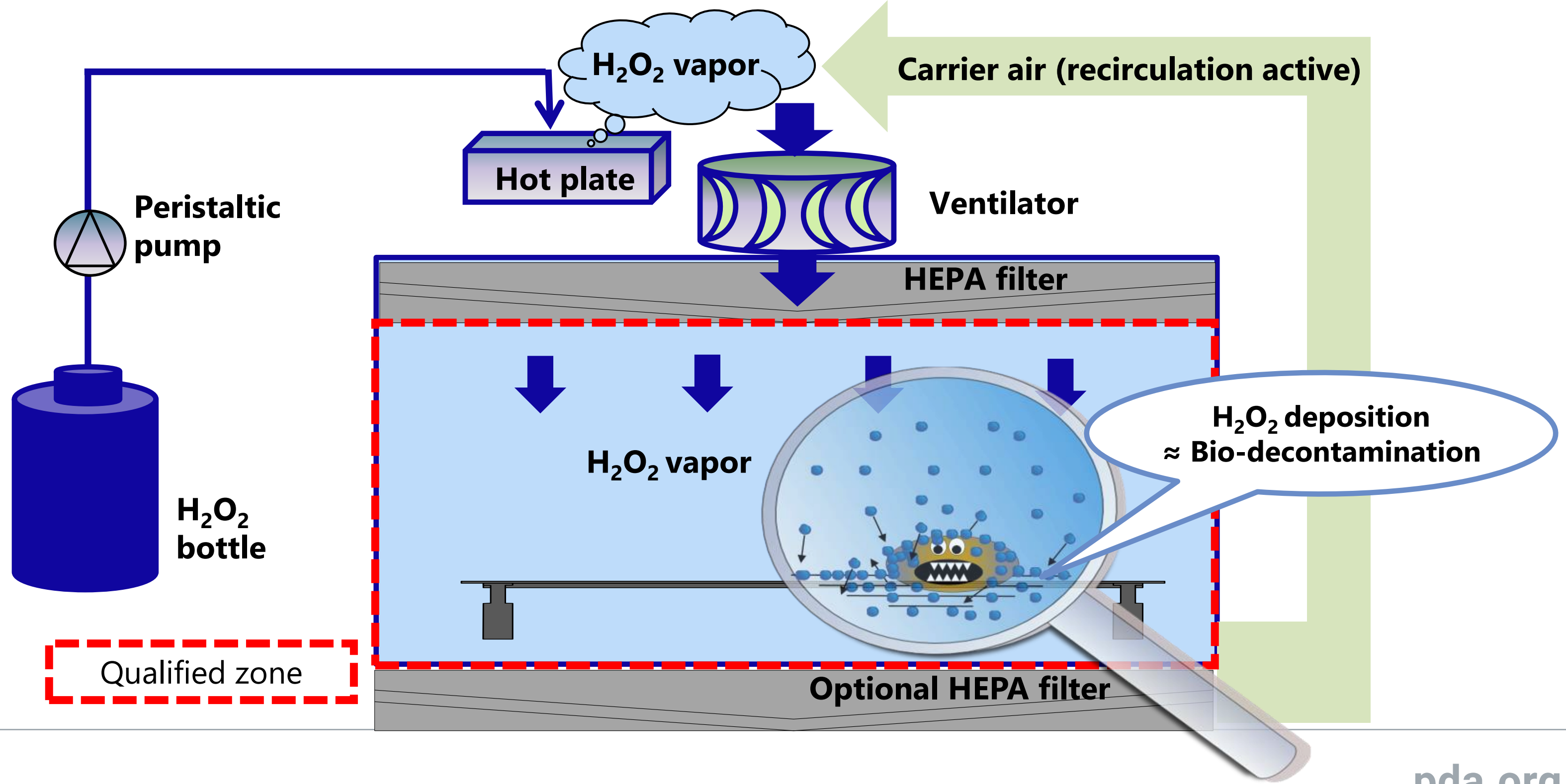
Deposition

Removal

…Different technical solutions

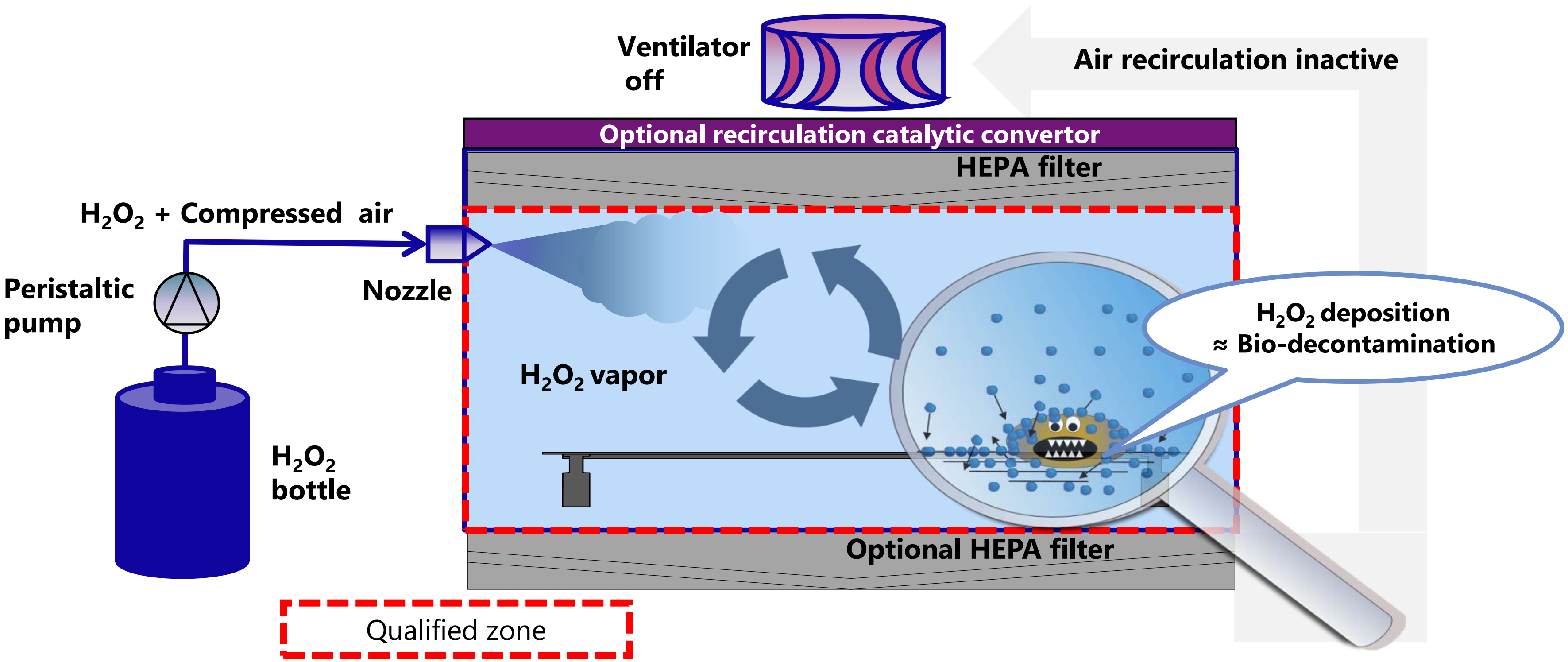
Hot plate evaporation

Example – SIS-700 System



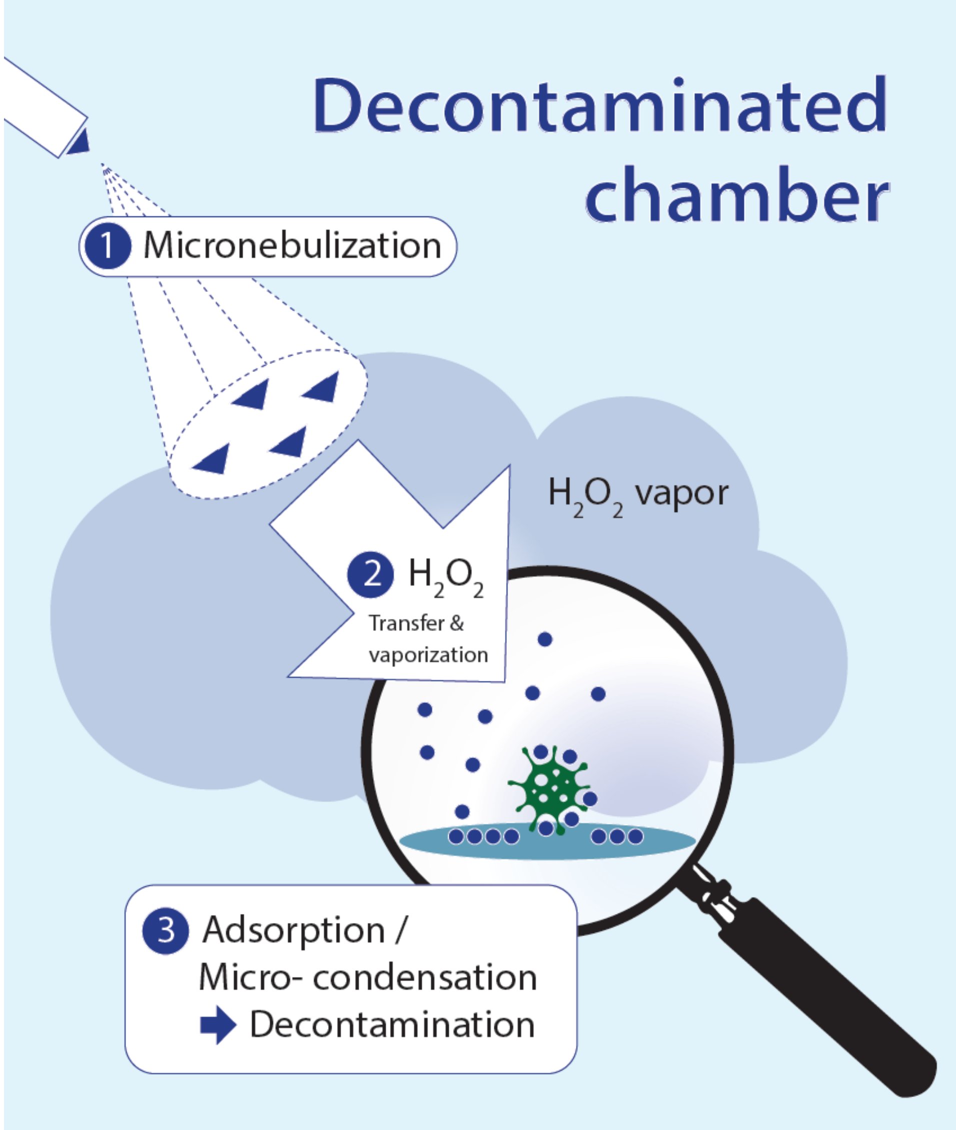
Evaporation by fogging

Example – skanfog - micro-nebulization

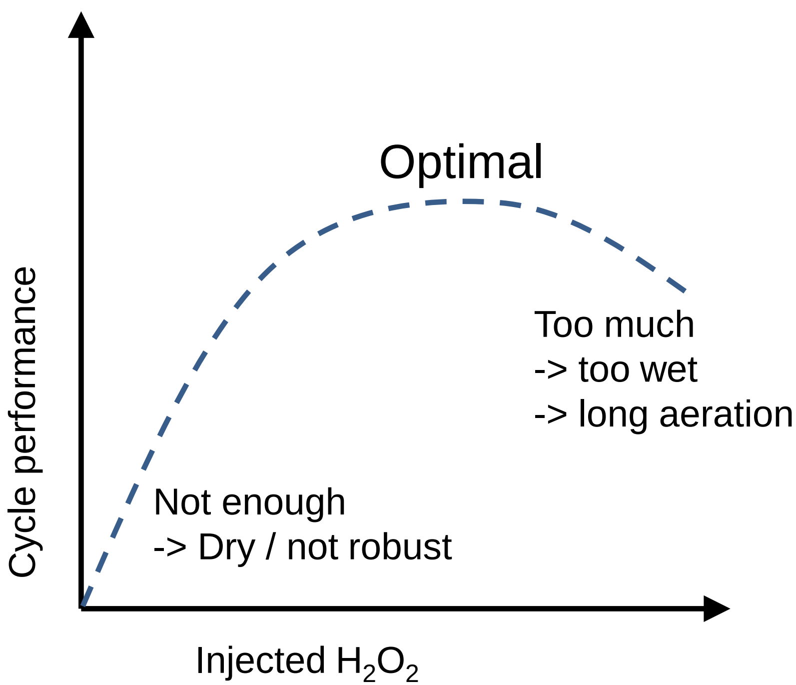


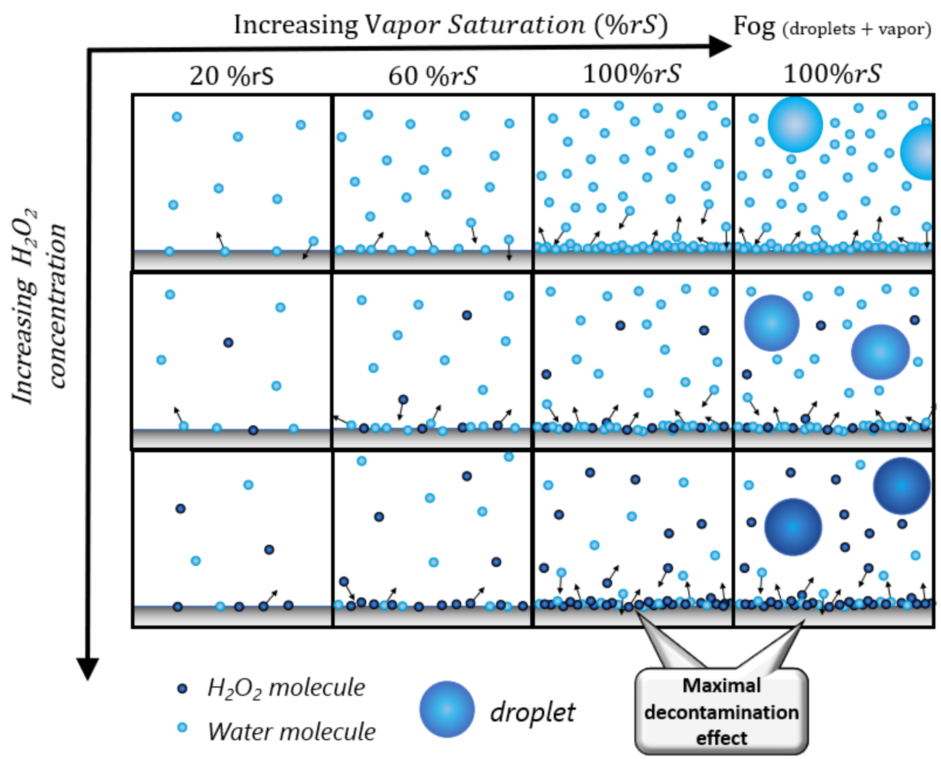
H_2O_2 Fogging

- Step 1 - Generation of micro-droplets
- Step 2 - Transfer and droplet evaporization
- Step 3 - Deposition of H_2O_2 on surfaces



H_2O_2 vapor conc. + Saturation vs. cycle performance





Fogging vs Hot plate

Fogging

- Robust and effective
- "Cold" vaporization
- Allows fast H_2O_2 injection
- Less H_2O_2 consumed
- Reduced HEPA filter exposure
- Ventilation not required
- Nozzle positioning
- Flexible and scalable
- Cycle times <1 hour possible

Hot plate

- Robust and effective
- "Hot" vaporization
- Slower H_2O_2 injection required
- Higher H_2O_2 consumption
- Full HEPA filter exposure
- Requires ventilation
- Vaporizer positioning
- Less flexibility/scalability
- Cycle times <2 hours possible

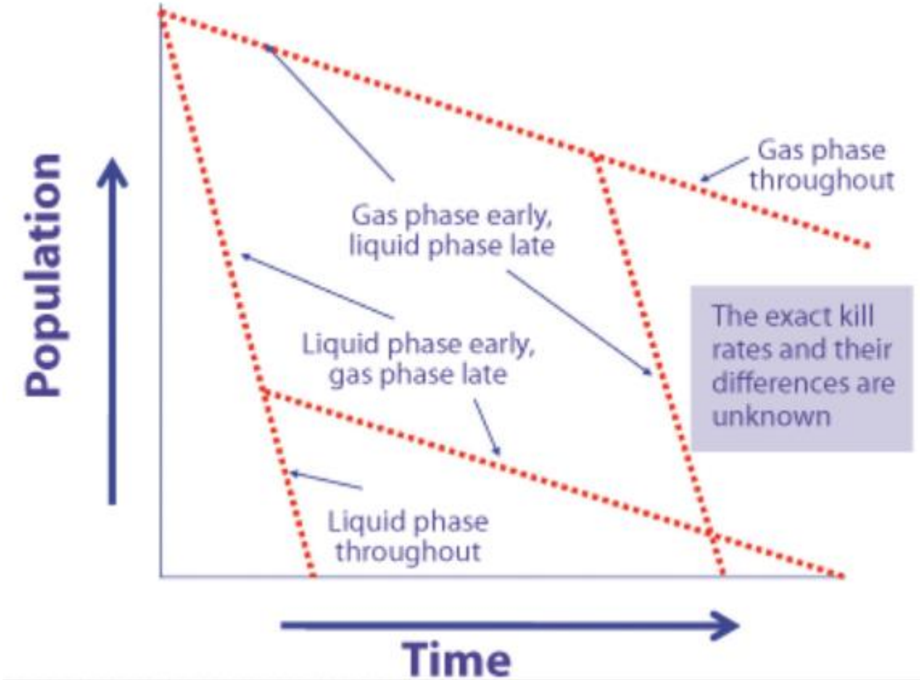
While the technology of vapor delivery is different, fundamentals remain the same!

Various technologies may offer benefits depending on the process needs

H_2O_2 bio-decontamination
"VPHP", "VHP", "aHP", "HPV", etc..

Bi-phasic process (limitations?)

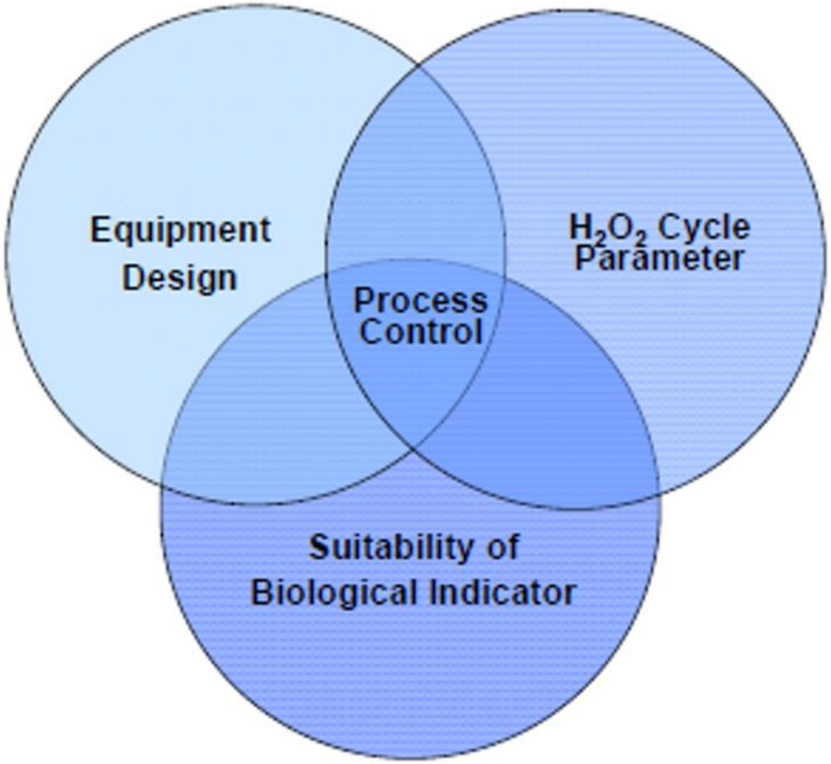
- Thanks to Bi-phasic (Vapor-Liquid) behavior of H_2O_2, the technology is so powerful
- Due to Bi-phasic (Vapor-Liquid) behavior, the technology is complex and difficult to master
- For its potency, many technical solutions exist … "dry vs wet", "vapor vs fog", etc..
- No standard "kill" conditions are defined



(1) Akers, J.; James P. Agalloco. Overcoming Limitations of Vaporized Hydrogen Peroxide. Pharmaceutical Technology 2013, 37 (9).

Process Control Strategy

- The same general principles apply for all H_2O_2 vapor phase bio-decontamination techniques
- Key Aspects:
  - Suitability of Biological indicator and other tools
  - Equipment design
  - Process expectations, QRM, CCS (deco effect, residual H_2O_2)
  - Justification of cycle parameters during cycle development and qualification/validation



H_2O_2 Cycle Control Strategy

Cycle Development

Best case efficacy tests
Loading pattern definition
Chemical indicator mapping
Temperature/humidity mapping
Worst case position study tests
Safety margins
Aeration time tests

Equipment manufacturing

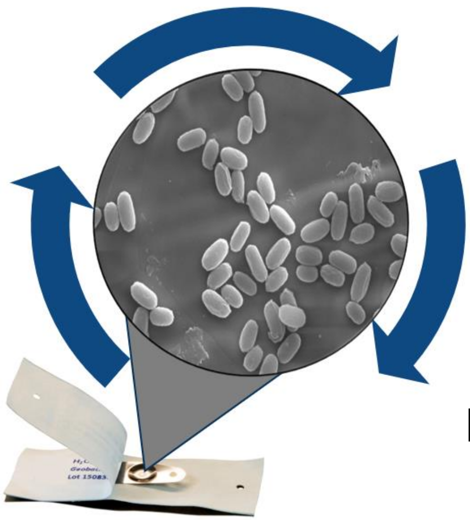
Manufacturing controls and tests
FAT -> delivery -> IQ, OQ, SAT

Equipment design

Fitting customer requirements
Quality by Design
Calculations/ Simulations
Know-how and expertise

Process control

Cycle control parameters
Process sensors
Process parameter alarms



Technology development

Process parameters
Control parameters
Process robustness
Material suitability
Component selection

Performance qualification

Microbial Qualification (MBQ)

Process Verification

periodic requalification (MBreQ)
In-process Parameter trending ~ Cycle Health

Biological indicators (BIs)

- Tools for evaluation of microbial inactivation processes
- BI consists of homogeneously distributed biocontamination on a metal carrier packed in permeable membrane

- Typical BIs for H_2O_2 decontamination
  - Spores of *Geobacillus stearothermophilus* (DSM5934 (=ATCC 7953)
  - BIs with excess of 10^4, 10^5 or 10^6 CFU/carrier
  - Carrier material - Stainless steel
  - Primary packaging - Tyvek®
  - Custom BIs can also be used

"BI is a characterized preparation of a specific microorganism that provides a defined and stable resistance to a specific microbial inactivation process" *(USP* <55>)

Biological Indicators (BIs)

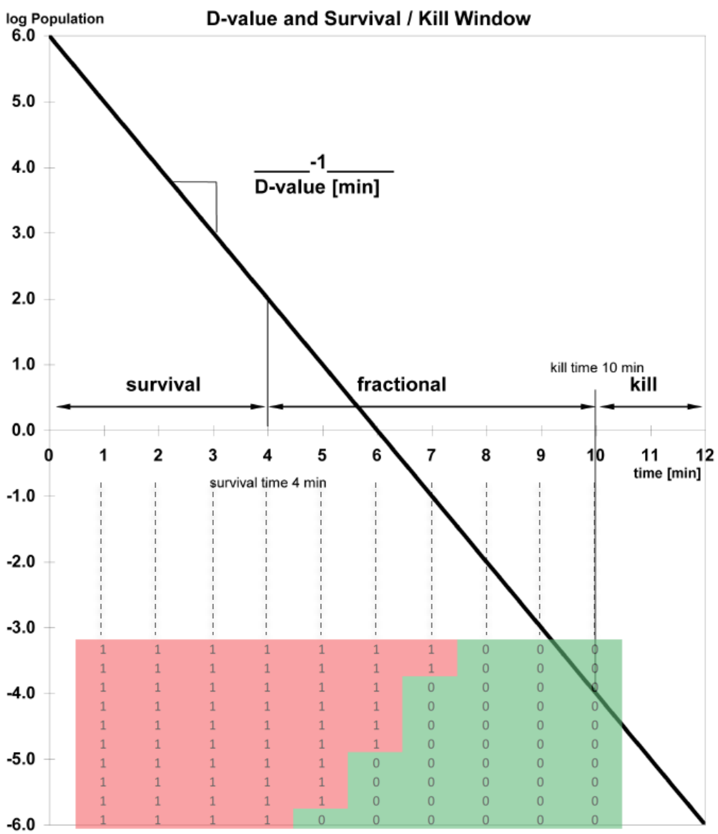
- BIs are the only tools capable to directly measure microbial inactivation
- Suitable BI is a corner-stone of any qualification/validation strategy for H_2O_2 bio-decontamination applied not only in isolators

" The bugs don't lie…
…If you stop using the microorganism as the actual measurement indicator, it starts to be inferential and not a direct measurement."
Rick Friedman (Deputy Director, office of Manufacturing Quality, CDER/FDA)
@ ISPE Aseptic conference 2022 regulatory panel

"The biological indicator provides a means to directly assess the sterilizing effect of the method in a manner not possible by physical measurements."
(USP<1229>)

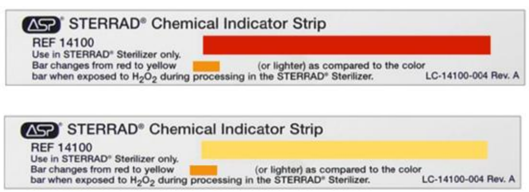
BI resistance and variability

- BIs do not have absolute resistance, it is a statistic
- Resistance of BIs is typically expressed as D-value
- D-value = the time needed to reduce viable population on a BI carrier by 90% (i.e. 1 log reduction) when exposed to bio-decontamination "kill" conditions
- For H_2O_2 standard "kill" conditions do NOT exist
- Resistances given by BI manufacturers in CoAs are informative only -> do not consider labeled D-value as your system D-value
- Importance of model behavior – within lot variability Lot should behave homogeneously, minimum of late positives



Chemical indicators (CIs)

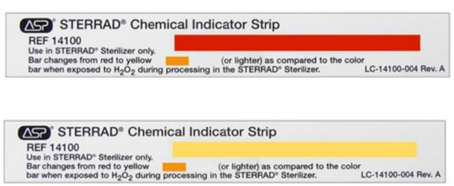
- Qualitative CIs play minimal (yet sometimes very useful) role
  - Immediate and simple readout (color change visible with naked eye)
  - Qualitative indication of H_2O_2 presence only
  - Weak information with regards to cycle effectiveness
  - Quick check of the decontamination homogeneity/ distribution
  - Can be used for troubleshooting, design optimization purposes



Enzyme Indicators – emerging quantitative CIs

- Enzyme Indicators (EIs) allow quantitative readout after the cycle
- “Best” CIs on the market
- High price and effort required compared to other CIs
- **More information / data can be collected with EIs, but BI’s remain the only proof**
  - What does the EI data mean?
  - Is the effort of collecting the data worth it?
  - What could be the use of it?
  - Hybrid strategies (BIs + EIs) are being investigated over the industry

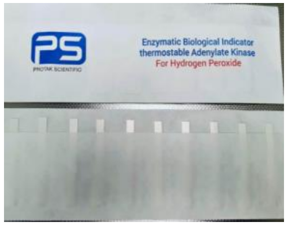
Chemical indicator evolution



Qualitative CIs

+

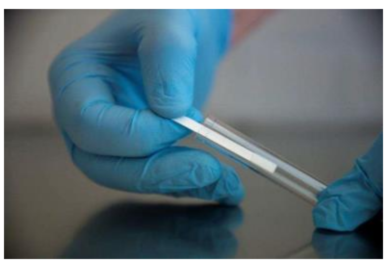
Semi-Quantitative CIs

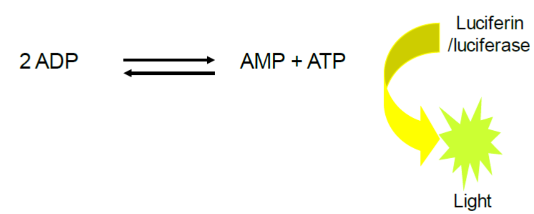


Quantitative CIs

Enzyme indicator technology principle

- **Sensing principle:** Degradation of thermostable enzyme by H_2O_2
- **Readout principle:** Light generated by chemical reaction catalyzed by the enzyme
- **Quantification:** Light measurement inside of a luminometer -> RLU (relative light units)
- **Interpretation:** Less Light generated = More enzyme degradation = More H_2O_2 (effect)





At scale considerable effort and cost

EI technology impressions

- EI response was showed to relate with BI inactivation
  - Correlation model was proposed and published in 2017[1]
    - 1 location within 1 specific system, 1 specific cycle recipe, 1 specific BI type and lot
  - Since then, a variety of publications were released

- EI seems not able to predict the BI behavior universally
- EI brings novel quantitative data relating to distribution of H_2O_2 and seems capable to augment cycle development studies

- Significant efforts and data are required to switch from informative to interpretable and actionable data

(1) McLeod, N. P.; Clifford, M.; Sutton, J. M. Evaluation of Novel Process Indicators for Rapid Monitoring of Hydrogen Peroxide Decontamination Processes. *PDA journal of pharmaceutical science and technology* **2017**, *71* (5), 393–404. DOI: 10.5731/pdajpst.2016.007435.

Inactivation rates of EIs and BIs may not change in synchrony

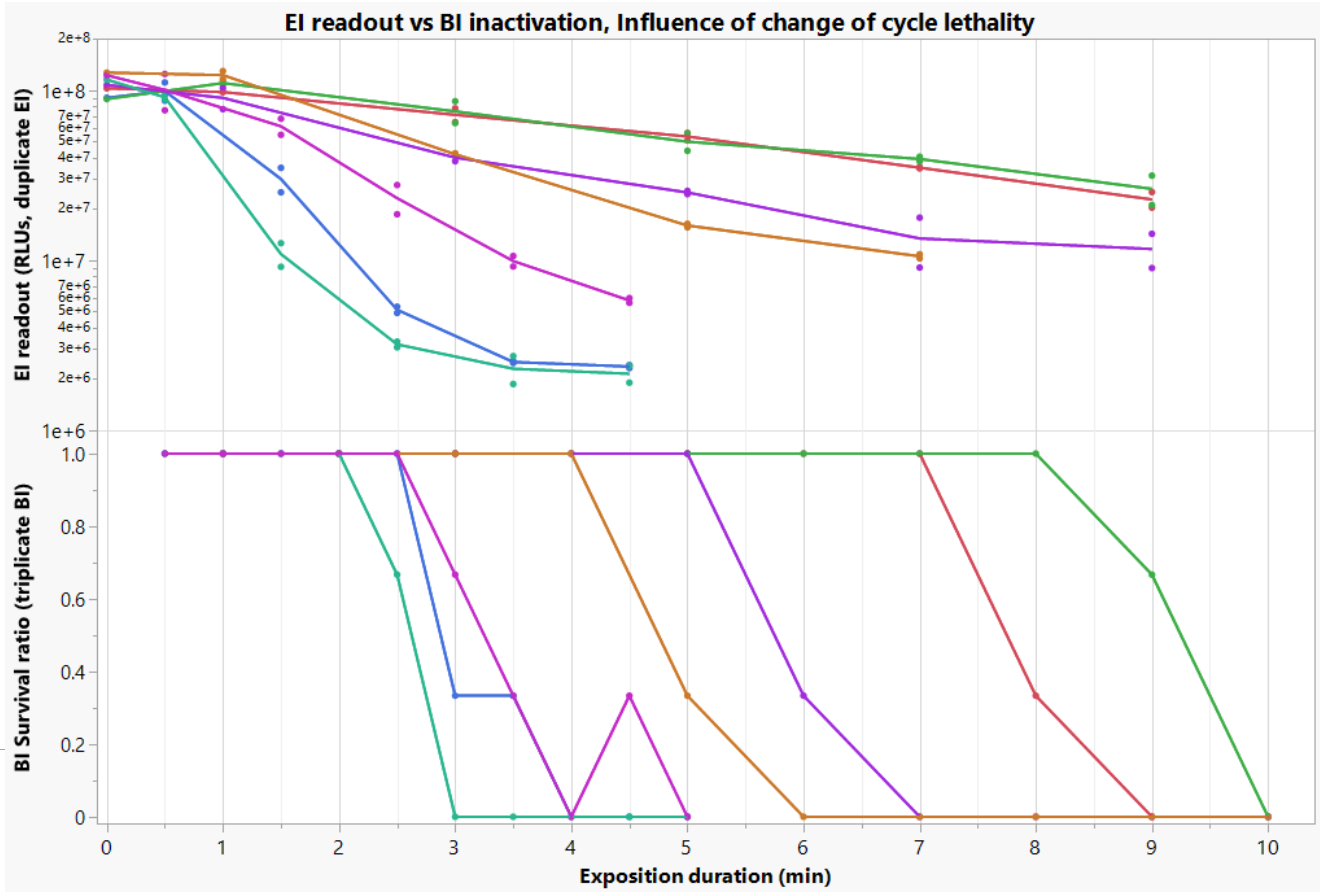
- **Example:** 1 technology, 1 BI lot, 1 system and sample location, variation of cycle lethality

- Changes of cycle lethality result in change of EI and BI inactivation rate

faster BI kill = faster EI inactivation

- EI readout (RLU value) corresponding to BI kill time changes with cycle lethality

1 Equation relating RLUs to log reduction is not applicable



Sensors: measurement of key in-process parameters

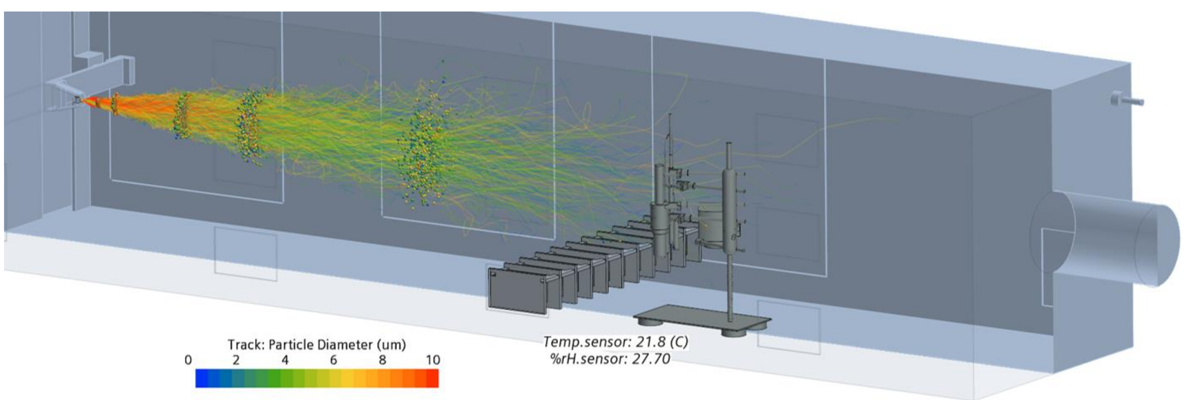
- Temperature
- Humidity
- H_2O_2 concentration (High and Low)
- (Relative saturation / Dew point)

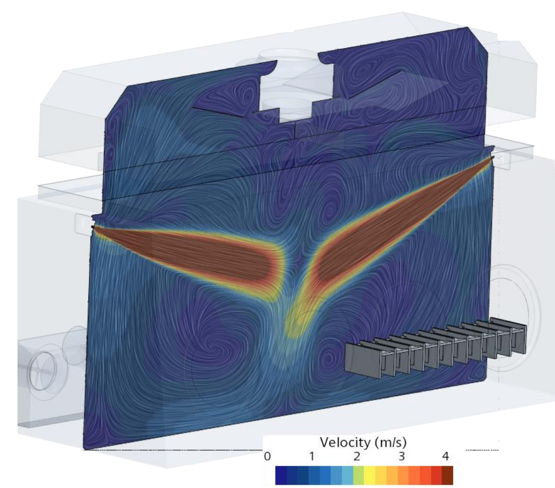
- There is no harmonized model relating key in-process parameters and H_2O_2 decontamination effect (i.e. BI kill / spore log reduction)

- Trending of in-process parameters allows for very good indication of cycle reproducibility -> **Cycle Health**

Bio-decontamination simulations (CFD)

- Computational capabilities are increasing exponentially
- CFD (Computational Fluid Dynamics) can now simulate and predict phenomena such as:
  - Airflow pattern and air-velocity fields (even for non-unidirectional/turbulent flows)
  - Spread of humidity and H_2O_2 over enclosure, even droplets
- In relation to novel sensorics and quantitative indicator tools (e.g. EIs) the simulations will increase in importance over time
- Can enable further process improvements on sustainability



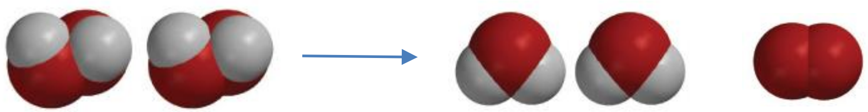


Residual H_2O_2 target

- Definition of Target H_2O_2 level
  - Typical target is <1ppm (or <0.5ppm) considering operator safety
  - Products may be extremely sensitive to oxidation and thus lower concentrations of 0.1ppm or even lower towards 30ppb are sometimes needed
  - Use spiking studies and trace H_2O_2 exposure tests to determine right H_2O_2 aeration target with regards to product quality

- Optimization of aeration duration
  - Technology selection, novel airflow concepts and catalysts enable extra short cycle times
  - Wrong selection of loading material may ruin any short cycle goal
  - Preliminary testing of H_2O_2 ingress into various materials will prevent any possible issues
  - Each plastic material is different!

H_2O_2 catalysts



2 H_2O_2 → 2 H_2O + O_2

H_2O_2 decomposes to harmless water (H_2O) and Oxygen (O_2)

- Degradation of H_2O_2 down to operator safe levels in a single pass through a catalyst
- Can greatly save time and energy requirements of the decontamination process.
- Terminal vs Recirculation catalysts
- Single-pass through catalysts able to degrade high levels of H_2O_2 are nowadays available

Common misconceptions

- H_2O_2 decontamination is a gaseous process
  - NO, H_2O_2 decontamination is two phase liquid-vapor process
- Condensation must be prevented during the cycle
  - NO, quickly reaching saturation and micro-condensation on surfaces makes inactivation quicker (also the surfaces above the dew point temperature become bio-decontaminated, but it typically takes longer)
- Condensation will damage the materials
  - NO, only materials tested to be persistent to H_2O_2 should be used in isolators and therefore this is not a concern (may be a concern for room bio-decontamination)
- Cycles able to get a “total kill” of 6 log BI (i.e. 8-9 log reduction) assure robust process
  - NO, H_2O_2 bio-decontamination has limited penetrability and therefore only suitable materials (e.g. non-porous) shall be used; surfaces need to be sufficiently clean
- D-values on BI certificates will apply for any H_2O_2 decontamination system
  - NO, D-values will differ system to system, the certified D-value may be used only to estimate lot-to-lot differences of a specific BI product/type, not much more

Thank you for your attention!

Questions?
Feel free to reach out via email.

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