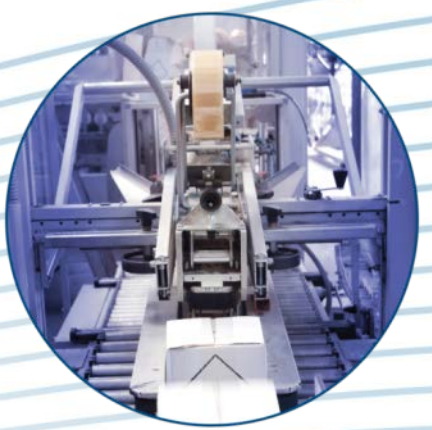
Connecting People, Science and Regulation®

Important Aspects of Sterilization, Cleaning and Disinfection, Gowning Procedures

by Guenther Gapp

18 October 2021



Presentation about Hot Topics in

- Sterilization & Depyrogenation
  - Moist Heat sterilization (Fo- Concept)
  - Dry Heat Depyrogenation
  - What can go wrong and common Audit findings
- Cleaning and Disinfection
  - Procedures and Best Practices
  - Common mistakes and audit findings
- Gowning Procedure to enter Grade A/ B room
  - Important points to consider

General Sterilization Methods

- Sterile Filtration: Products for Aseptic Filling
  - Liquids, Vent Filters - Air
- Steam (Autoclave and SIP)
  - Solid, liquid components, equipment
  - Equipment (e.g. filling nozzles)
- Dry Heat
  - Glassware, also for depyrogenation
- Radiation
  - Heat sensitive components
- Gas: Ethylenoxide
- VHP : Vaporized Hydrogen Peroxide (surface Sterilant)

Photo courtesy of Meissner Filtration Products

Photo courtesy of Wayne Metal Products Inc.

PDA Technical Reports to consider: TR No 1

Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control

Technical Report No. 1 (Revised 2007)
Supplement
Vol. 61, No. S-1

PDA℠
Parenteral Drug Association

PDA Technical Reports to consider: TR No. 48

PDA Technical Reports to consider : TR 61

Technical Report No. 61

Steam In Place

Definitions (from PDA TR 61)

Sterilization

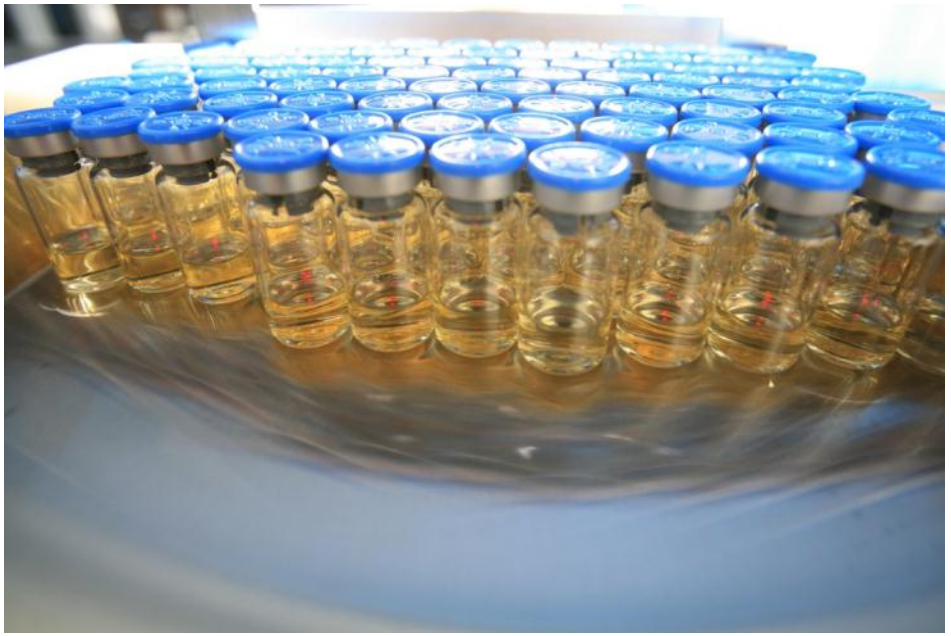
A process used to render a system free of viable microorganisms with a specified probability.

Definitions and Concepts

Sterile

Free from living organisms, especially microorganisms

Merriam-Webster definition



Definitions (from PDA TR 61)

Sterility Assurance Level (SAL)

Probability of a single viable microorganism remaining after SIP.

Note: The term SAL uses an assumed quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than an SAL of 10^{-3} ***(10)***.

Definitions (from PDA TR 1)

Overkill Design Approach: A sterilization design approach where minimal information is required about the product bioburden. A worst-case bioburden assumption is used to determine the delivered lethality needed to achieve a PNSU of 10^{-6} on or in the items being sterilized. When using this approach, the qualification program must demonstrate that both the F_{BIO} and F_{PHY} are greater than 12 minutes.

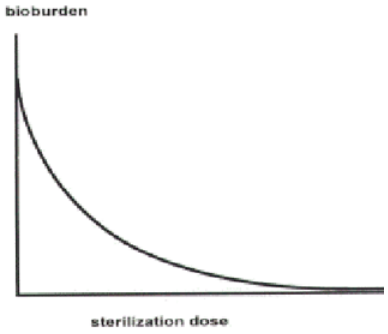
Definitions and Concepts

FDA 2004 & EU Annex 1 Revision

Overkill sterilization process- A process that is sufficient to provide at least a 12 log reduction of microorganisms having a minimum D value of 1 minute.

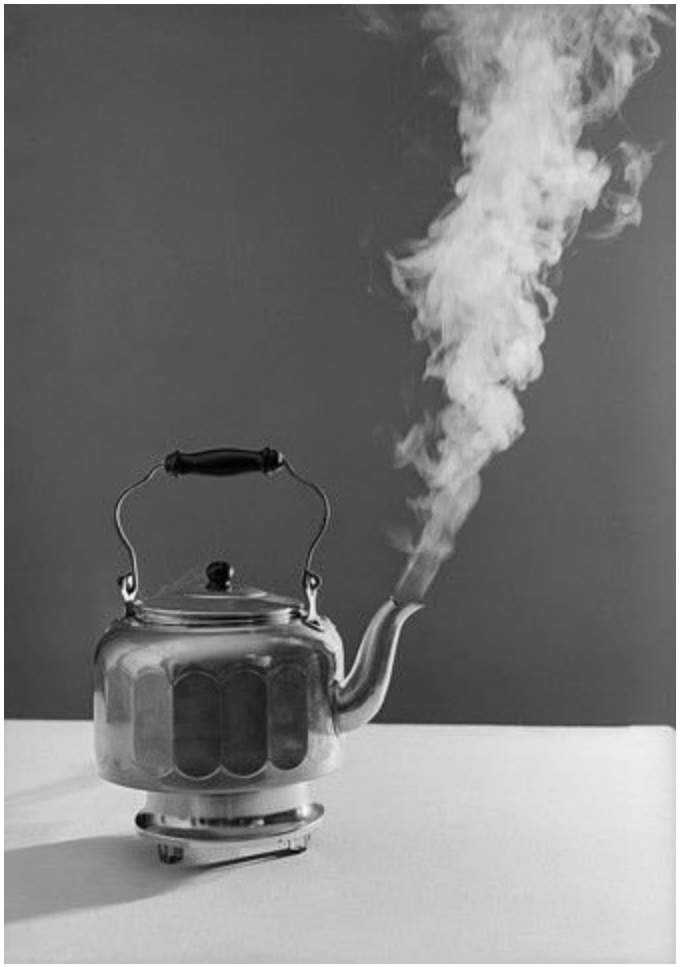
SAL

- Terminally Sterilized – 10^{-6}
- Des not apply to Aseptic Processing



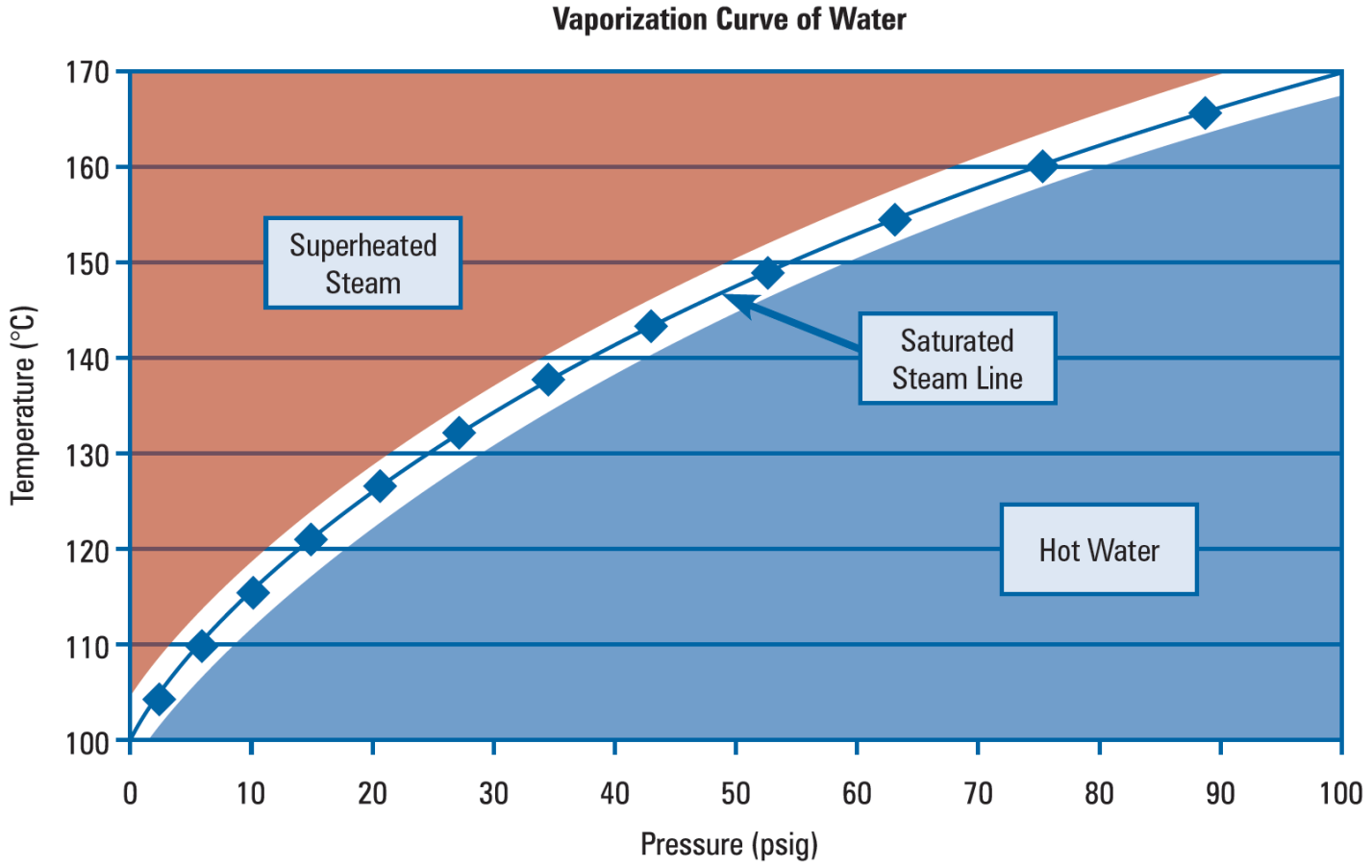
Steam – Moist Heat Sterilization

- Steam is water in the vapor phase
- As water changes from liquid to vapor a substantial amount of energy must be added
- When steam contacts an object at a lower temp, this energy (= latent heat) is given up as the vapor turns back to a liquid (condenses)
- The large exchange of heat and transfer of moisture to an object by steam accounts for its biocidal activity



PDA TR 61: (1 bar = 14,5 psig)… „Saturated Steam“

Figure 3.2-2 Optimal Heat Transfer Curve



PDA TR 61: … latent heat transfer: energy is transferred

3.2 Mechanisms of Lethality

The mechanism of microbiological lethality for steam in place systems is the thermal destruction of microorganisms by direct contact with the sterilizing medium (steam). The mechanism of heat transfer is conduction where the transfer of energy occurs from latent heat. As with other saturated steam sterilization methods, the rate of microbial destruction under conditions of constant temperature progresses logarithmically over time.

The kinetics for these complex reactions are best represented as a First Order chemical reaction. This means that there is a linear relationship between the logarithm of the number of surviving microorganisms and the time of exposure (see **Figure 3.2-1**).

- Thermal destruction by direct contact
- Transfer of energy occurs from latent heat
- Important is a prevention of air traps (no steam contact)

F-Values – Moist Heat

- F-Value (Lethality Factor):

A measurement **of sterilization effectiveness** *(like the Killrate/ Heat-Effect*) that is expressed as $F_{(Tref,z)}$ that is the calculated equivalent lethality, in terms of minutes at a reference temperature (Tref), delivered by a sterilization cycle to an item.

* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

The $F_{physical}$ –Value (= sum of lethalities)

- $F_{physical}$ value (F_{PHY}) is calculated by integrating (summing) the lethal rate over time:

$$F_{Tref} = d(\sum L)$$

Where:

d = the time increment between each temperature reading (*Note GG: typically 1 min time interval)*

L = the lethal rate calculated for each temperature reading

F_{PHY} for Tref of 121.1˚ C is expressed as $F_{121.1^\circ C}$ (Fo)

* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

Lethal Rate Calculations

- Lethal rate (in “Minutes”) is calculated by the following formula:

- $L_{(Tref,z)} = 10^{(T-Tref)/z}$

  T= Temperature of the item being heated

  Tref= Reference temperature (Usually 121.1˚ C)

  z= z-value of the challenge organism (or 10˚ C if not known)

* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

Lethal Rate: Examples

$$L = 10^{(120\text{-}121{,}1^{\circ}\,C/10)} = 10^{-0.11} = 0.78$$

Thus, one minute at 120° C is equivalent to 0.78 minutes at 121° C

$$L = 10^{(121.1\text{-}121{,}1^{\circ}\,C/10)} = 10^{0} = 1.00$$

Thus, the Lethality for one minute at 121.1° C is 1 minute

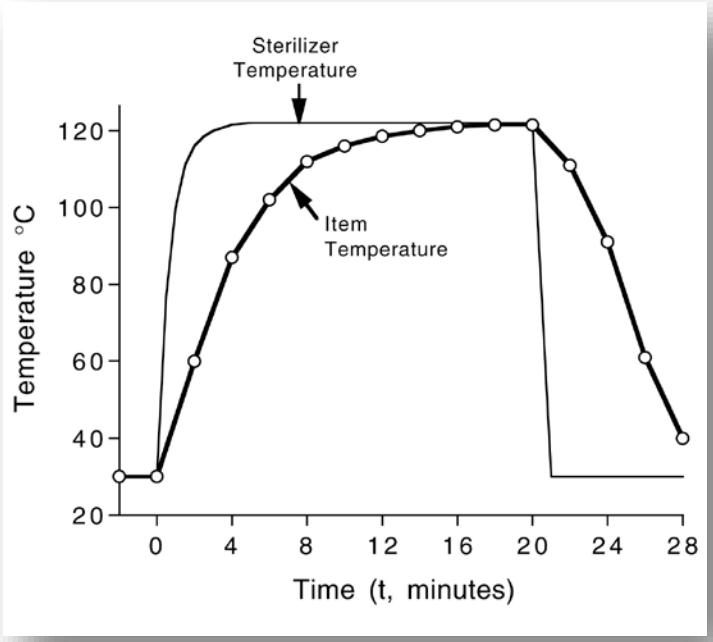
* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

PDA TR 61: Fo is the equivalent lethality at specific reference conditions

F_0

A term used when the *specific* reference conditions of $T_{ref} = 121.1°C$ and $z = 10°C$ are used to calculate the equivalent lethality. For example, when the z-value of the BI is 10°C, a cycle with an $F_{(T=121.1°C, z=10°C)}$, or F_0, equal to 8 minutes is equivalent (in terms of delivered lethality) to a square wave cycle of 8 minutes at 121.1°C. A square wave cycle that provided an exposure of 25.9 minutes at 116°C would also yield an F_0 of 8 minutes.

WHY do we need a Fo- value and reference model ?



D- Value : time for 1 log reduction

- D-Value: The time in minutes required for a one logarithm, or 90%, reduction of the population of microorganisms used as a biological indicator under specified lethal conditions.
- For steam sterilization, the D-value should always be specified with a reference temperature, D_T. For example, a BI system with a $D_{121^\circ C}$ = 2.5 minutes requires 2.5 minutes at 121˚ C to reduce the population by one logarithm.
- Typical bio-indicator for moist heat sterilization is Geobacillus stearothermophilus

* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

z-value: temp. change for a D value Change Factor (10x or 1/10)

- z-value is defined as the number of degrees of temperature change necessary to change the D-value of a biological indicator (BI) by a factor of 10. The z-value is a component of the F-value calculation.

- A z-value of 10˚ C is generally used in routine steam process design and evaluation.

* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

F_0 –Value

- F_0 value (F_{PHY}) is calculated by integrating (summing) the lethal rate over time:

$$F_0 = d(\sum L)$$

Table 3.2-1 Example Lethality Rates (F0 per Minute) at Various Process Temperatures

°C	F_0 Per Minute
100.0	0.008
105.0	0.025
110.0	0.078
115.0	0.245
120.0	0.776
121.1	1.000
125.0	2.455
130.0	7.762
135.0	24.547

Lethality for 1 min at a specific temperature

* Source: PDA Technical Report No.1, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control, 2007

Example for better understanding : Autoclave cycle and resulting Fo value

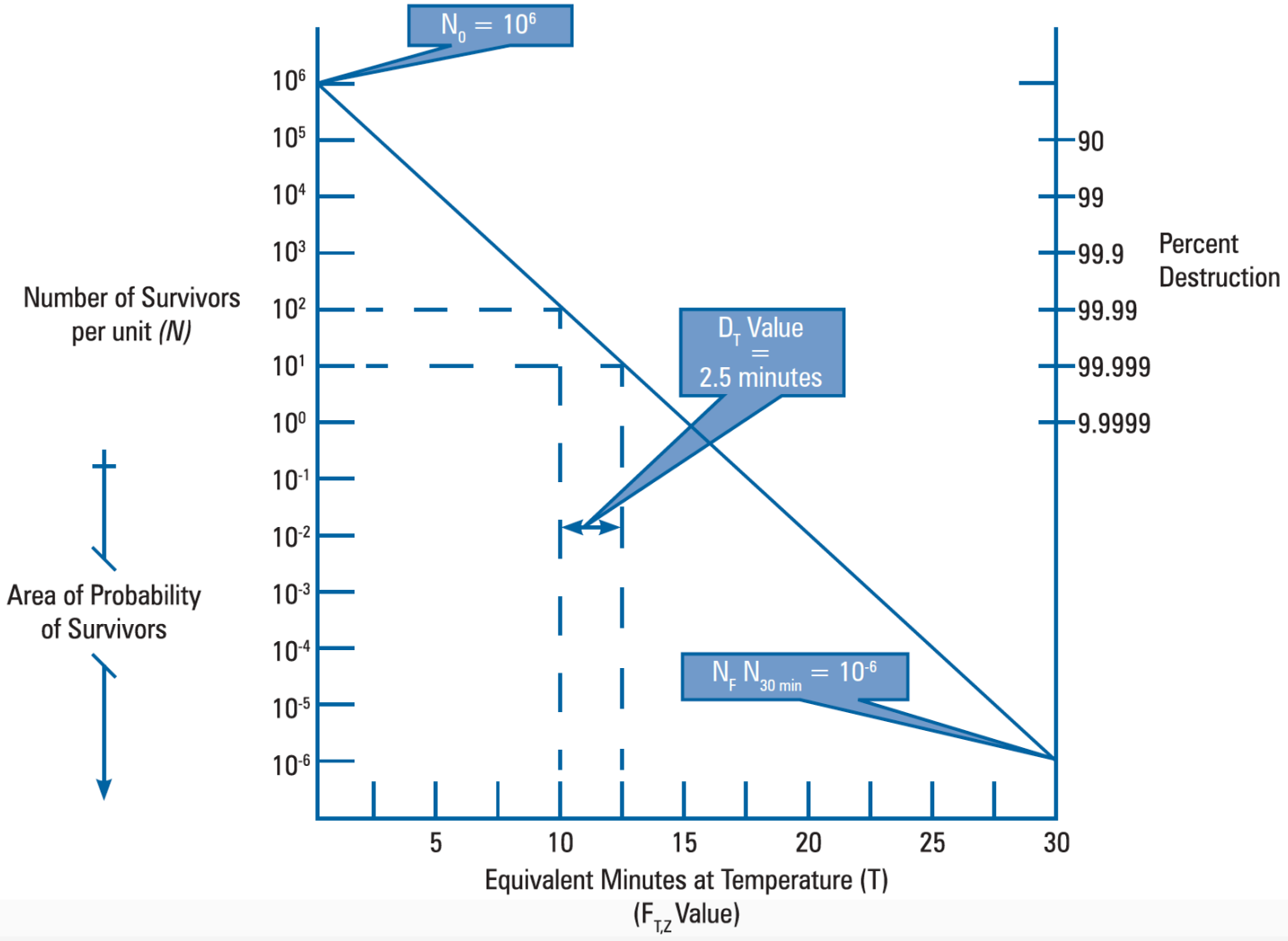
Autoclave Cycle: Calculation of Sterilization cycle

Time	Temperature	Lethal Rate (L)	Fref d x L	Accumulated L
12:02	110	0,08	0,08 (1 min x 0.08)	0,08
12:03	120	0,78	0.78 (1 min x 0.08)	0.86
Start 12:04	121.1	1	1 (1 min x 1)	1.86
12:05	121.5	1.10	1.10 (1 min x 1.10)	2.96
12:06	122.0	1.23	1.23 (1 min x 1.23)	4.19
12:07	122.5	1.38	1.38 (1 min x 1.38)	5.57
12:08	122.0	1.23	1.23 (1 min x 1.23)	6.8
12:09	121.5	1.10	1.10 (1 min x 1.10)	7.9
12:10	121.0	0.98	0.98 (1 min x 0.98)	8.88
12:11	122.0	1.23	1.23 (1 min x 1.23)	10.11
12:12	122.0	1.23	1.23 (1 min x 1.23)	11.34
12:13	121.5	1.10	1.10 (1 min x 1.10)	12.32
12:14	121.0	0.98	0.98 (1 minx 0.98)	13.3
12:16	122.0	1,23	1,23 (1 min x 1.23)	14.28
12:17	121.5	1.10	1.10 min	15.38
12:18	121.5	1.10	1.10	16.48
End 12:19	121.5	1.10	1.10	17.58
12:20	120	0,78	0.78 (1 min x 0.78)	18.36
12:21	110	0,08	0,08 (1 min x 0.08)	18.44 min
			F0 = 18.44 min	

$F_0 = d(\sum L)$

PDA TR 61: 12 log reduction / D 2.5 min → Fo 30 min

Figure 3.2-1 Microbial Survivor Curve



Sterilization Effect with a Fo 12 min

(Note: this is would be no overkill sterilization, only for a D- value of a Biological Indicator of 1 min)

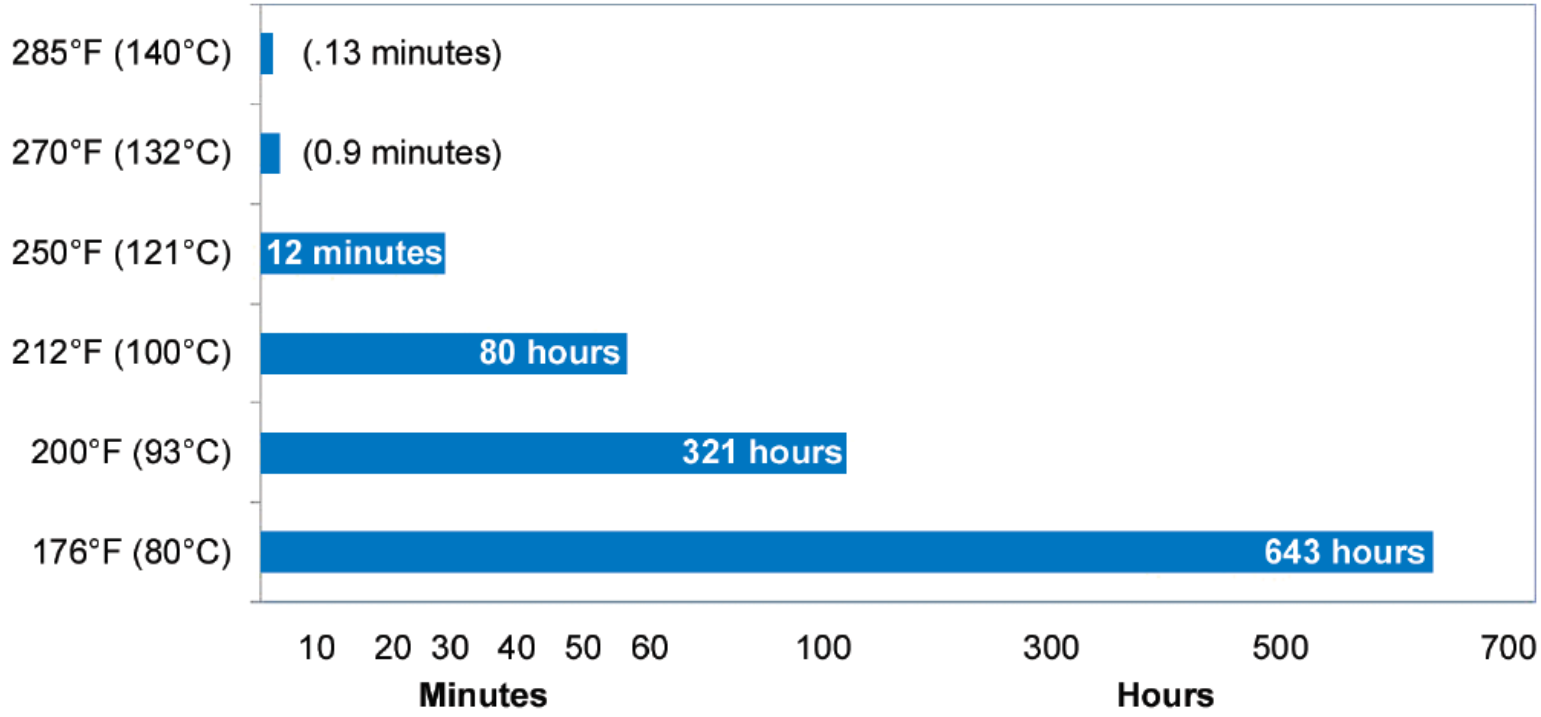


Figure 2. Sterilization time versus temperature.

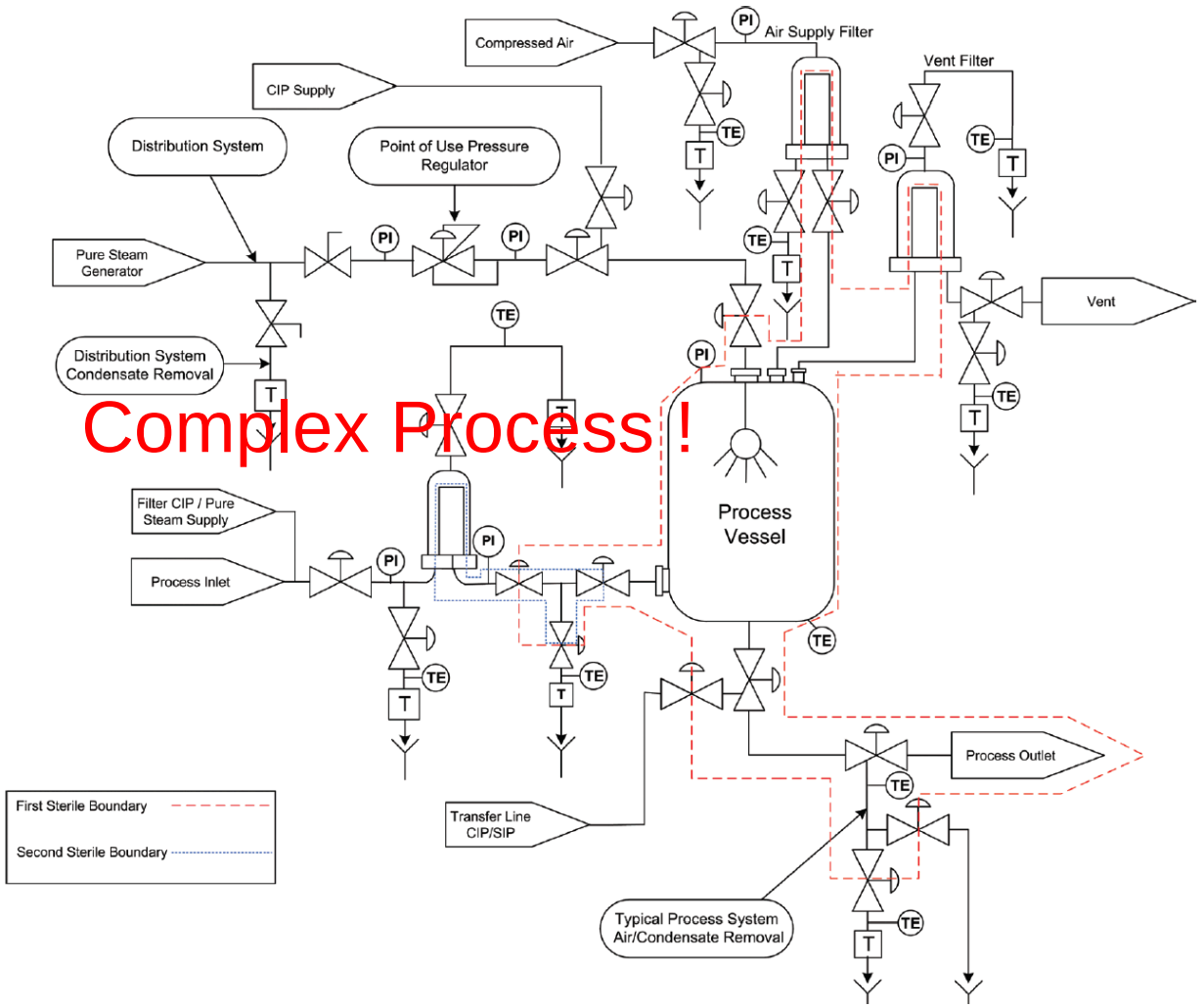
Overkill Approach/ Model

- Common definition: Fo > 15 min
- But also defined as a sterilization cycle which provides a > 12-log reduction of the bioburden
- But: Biological Indicator of D- value 1,5 in – would require even a Fo > 18 min for a 12 log reduction.

- Note : D- value might be lower within product solutions. A 12 log reduction of BI is required (Finally might be lower than Fo of 15 min).

SIP ….Steam in Place (TR 61)

Figure 4.2-1 Example of Steam Distribution and Process Tank Layout



Autoclave /SIP

- Common Audit findings :

Loading configurations in validation and routine manufacturing are not 100 % identical

Parts wrapped within aluminium paper , ineffective air removal

Thermocouples / - controls not positioned at worst- case positions

Hot Topics for Autoclave /SIP

- Steam Quality
- Non- condensable gases
- No Temperature & Pressure correlation
- Evacuation cycles (e,g. within hoses or wrapped material)
- Microlab: autoclave (waste treatment should be separate autoclaves/ media preparation- do not overcook)

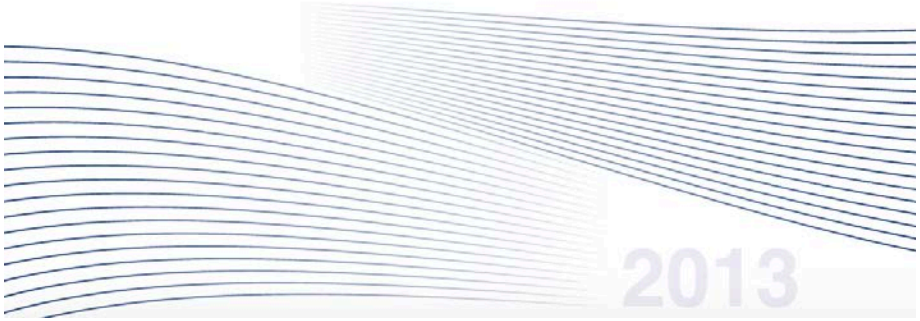
…

Dry Heat Sterilization / Depyrogenation

PDA Technical Report to consider

Technical Report No. 3 (Revised 2013)

Validation of Dry Heat Processes Used for Depyrogenation and Sterilization



Validation of Dry Heat Processes Used for Depyrogenation and Sterilization

Technical Report No. 3 (Revised 2013)

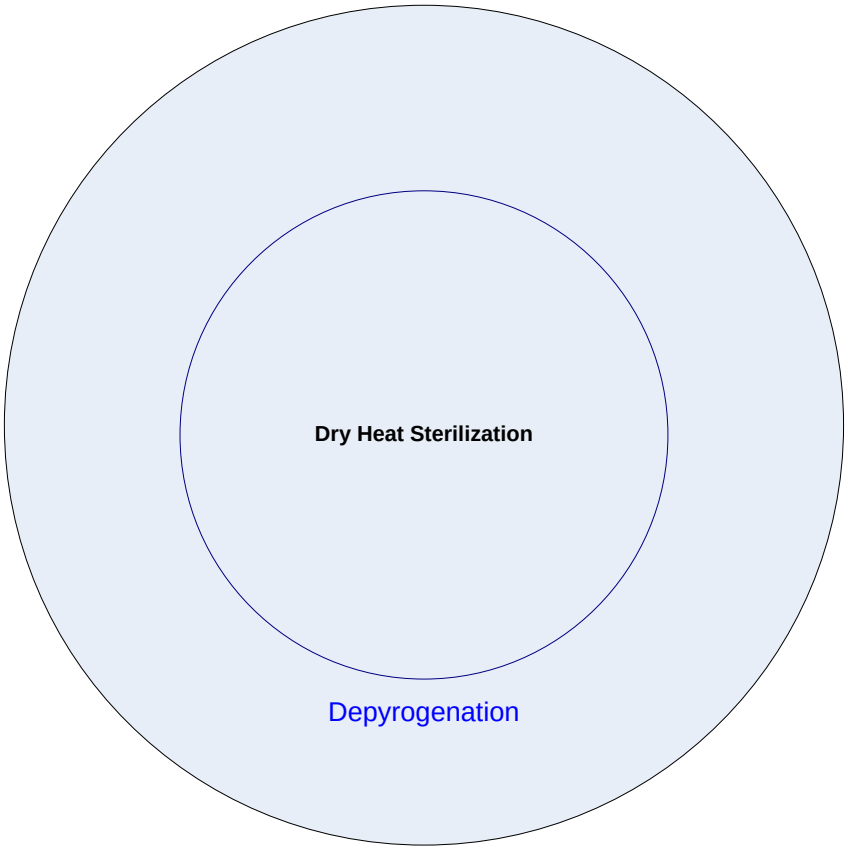
ISBN: 978-0-939459-56-8

Dry Heat Sterilization

- Uses only hot air
- Requires higher temperatures than moist heat
- Can be batch process (oven) or continuous (tunnel)

Dry Heat Depyrogenation and Sterilization

- Dry heat processes can provide sterilization or both depyrogenation and sterilization



F- value (Dry Heat) / TR 3

F-Value (Lethality Factor)

A measurement of process effectiveness. F_z^{Tref} is the calculated equivalent lethality (using a specified z-value) for a sterilization process, in terms of minutes at a reference temperature (T_{ref}), delivered by a sterilization process to an item.

F_H

A term used when the specific reference conditions of T_{ref}=160°C and z=20°C are used to calculate the equivalent lethality. For example, when the z-value of the BI is 20°C a process with an $F_{(T=160°C,\ z=20°C)}$, or F_H, equal to 8 minutes is equivalent (in terms of delivered lethality) to a square wave process of 8 minutes at 160°C. A square wave process that provided an exposure of 45.2 minutes at 145°C would also yield an F_H of 8 minutes.

Dry Heat / TR 3

3.3.1.1 F_H-Value for Sterilization

F_H is a measure of heat input. The F_H concept is comparable to the F_0 concept for moist heat sterilization and references lethality to equivalent times at 160°C. Other reference temperatures can also be considered, but 160°C is primarily used ***(30)***. F_H values are shown in units of minutes or seconds, and the calculations of F_H use the same equations as the calculations of F_0 (**Equation *3***).

F_H is a term used to model exposure time to dry heat. By definition, F_H is expressed by a reference temperature so that it truly represents the equivalent exposure time, in terms of lethality, at that reference temperature. Since routine operational processes are not generally square wave processes (i.e., the load does not come up to temperature instantaneously, remains at the precise set point throughout the exposure phase, and then cools down instantaneously), the *z*-value, or temperature coefficient, is used in the model to calculate the equivalent lethality at different temperatures during the cycle.

Theoretical F_H values can be calculated using the following parameters:

- *z*-value = 20°C
- T_{ref}-value = 160°C

Depyrogenation (TR 3)

3.1 Depyrogenation

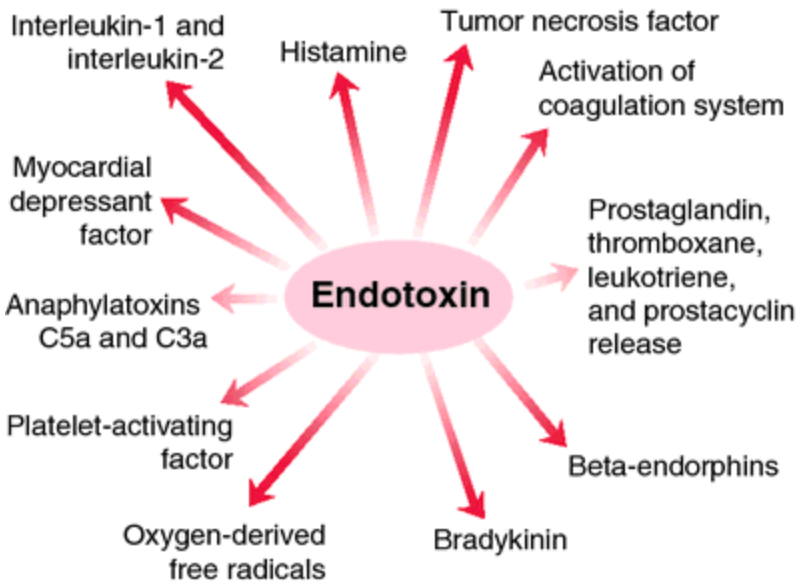
There are a number of depyrogenation methods used to inactivate or remove bacterial endotoxins ***(12,13)***. Dry-heat depyrogenation is the primary method used for the inactivation of bacterial endotoxins by thermal destruction. Dry-heat depyrogenation ovens or tunnels have been used for the depyrogenation of heat-resistant materials like glassware, metal equipment, instruments, containers, and heat stable chemicals ***(7,12–15)***. The development and use of the *Limulus* amebocyte lysate (LAL) assay has also provided a means of assessing the performance of dry-heat endotoxin inactivation on a quantitative basis ***(11,16,17)***.

The selected temperature and exposure time should be appropriately validated to demonstrate that the dry-heat depyrogenation process delivers an adequate and reproducible level of endotoxin reduction when operated routinely within the established tolerances.

Since dry heat is frequently employed to render glassware or containers free from detectable endotoxins as well as inactivate viable microbes, an endotoxin challenge, where necessary, should be an integral part of the validation program, e.g., by inoculating one or more of the articles to be treated with 1000 or more USP endotoxin units (EU) of standardized lipopolysaccharide ***(3)***.

Depyrogenation

Endotoxins are fever producing substances commonly found in the cell wall of certain Gram negative bacteria.



Depyrogenation:

- The destruction or removal of bacterial endotoxins.
- A depyrogenation process should demonstrate at least 99.9% or a 3-log endotoxin reduction.

Glossary (PDA TR 3)

Depyrogenation

The destruction and/or removal of bacterial endotoxins. A depyrogenation process should demonstrate at least 99.9% or a 3-log endotoxin reduction.

Depyrogenation tunnel / TR 3

Figure 4.1.3-1 Continuous Convection Tunnel

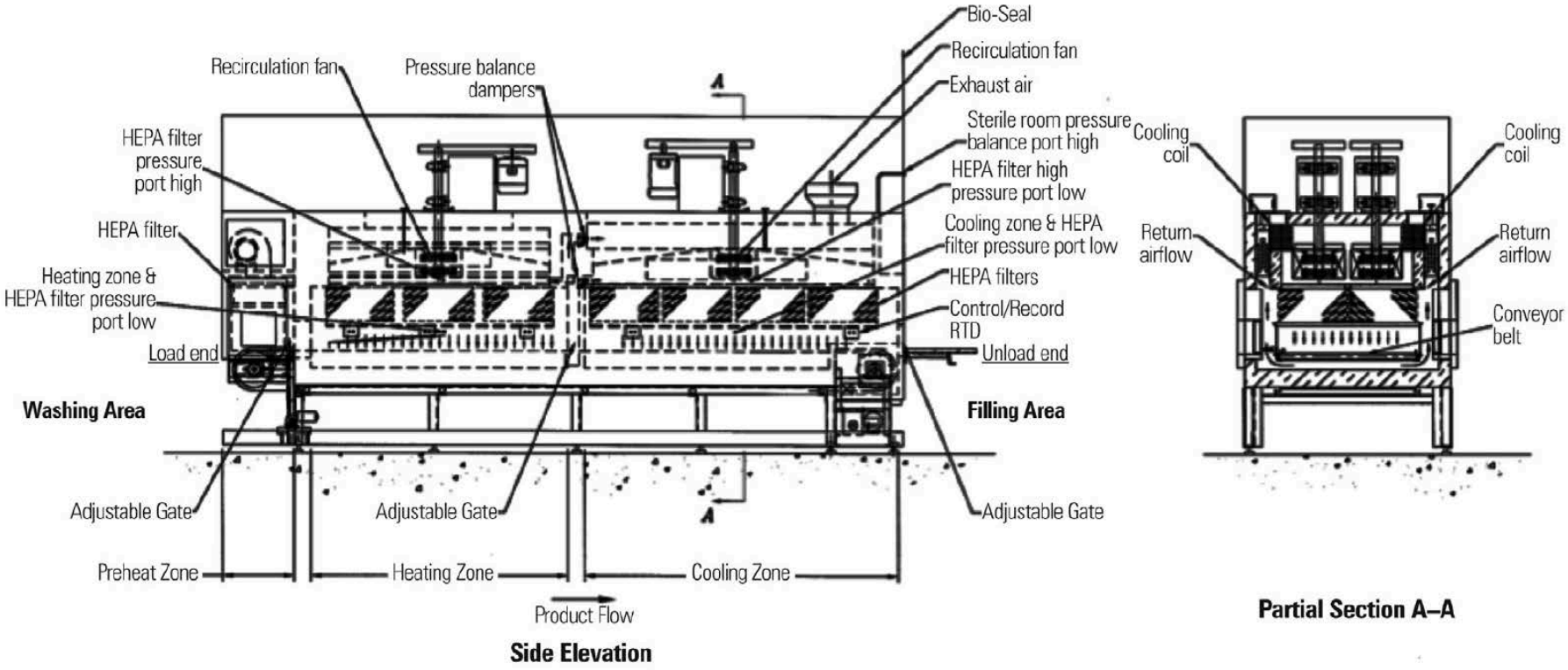


Image Courtesy of Despatch Industries

Questions to audience : How to prepare/ sterilize ... for aseptic filling ?

- Glass- ware (vials/ ampoules)
- Rubber stoppers for vials
- SST piston pump
- EM controls (e.g. settle plates)
- Forceps and scissors
- Glass – Syringes within Tubs
- Product contact surfaces (filling nozzles)
- Indirect Product contact surfaces (stopper bowl, tracks)

Cleaning and Disinfection Aspects

Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities

Technical Report No. 70

ISBN: 978-0-939459-77-3

PDA Parenteral Drug Association

Definitions According to PDA TR70

Detergent
A synthetic wetting agent and emulsifier that can be added to a solvent to improve its cleaning efficiency.

Disinfectant
A chemical or physical agent that reduces, destroys, or eliminates vegetative forms of harmful microorganisms but not spores.

Sporicide
A compound that destroys all vegetative microorganisms and bacterial and fungal spores.

Sanitize
To make physically clean and to remove and destroy, to the maximum degree that is practical, agents injurious to health.

Sterile
The absence of viable microorganisms.

Sterilization
A process by which something is rendered sterile (i.e., moist heat, dry heat, chemical, irradiation); normally validated at 10^6 organism reduction.

Definitions According to PDA TR70

Contact Time

The minimum amount of time that a sanitizer, disinfectant, or sporicide must be left in complete (wet) contact with the surface to be treated in order to be effective.

- **Sanitizers**

  Sanitizers provide minimal reduction in thirty seconds to ten minutes and are often used for low levels of vegetative microorganisms. The type of sanitizer will dictate the appropriate contact time required. Alcohol is an example of a commonly used sanitizer.

- **Disinfectants**

  Disinfectants exhibit a higher level of efficacy than sanitizers, and their kill is dependent on the inoculums and the contact time. Disinfectants will typically kill vegetative microorganisms with the exception of spore-forming microorganisms. Examples include quaternary ammonium compounds and phenolics.

- **Sporicides**

  Sporicides provide up to a total kill depending on the inoculums and the wet contact time and will kill bacterial spore formers as well as mold. Products commonly used today include bleach, hydrogen peroxide, and a mixture of hydrogen peroxide and peracetic acid.

PDA TR 70

4.2 Regulatory Inspections

Due to their importance and direct impact on manufacturing operations, the cleaning and disinfection programs have been and continue to be a focus during regulatory inspections. Key components of any cleaning and disinfection program, which are often reviewed during inspections, include the following:

- Qualification of suppliers and agents
- Cleaning and disinfection methodologies
- Decision to use ready-to-use vs. ready-to-prepare chemical agents as well as the quality of water to be used (if needed) during their preparation
- Process used for sterile filtering of antimicrobial chemical agents
- Sterilization and storage of antimicrobial chemical agents used in aseptic processing areas
- Sterilization and storage of cleaning equipment (sprayers, buckets, mop heads, and mops)
- In-use expiration dating of antimicrobial chemical agents
- Rotation of agents
- Training, qualifications, and responsibilities of personnel and supervisors
- Frequency of cleaning and disinfection
- Contact times (wetted period)
- Method for addressing residuals
- Documentation for cleaning and disinfection
- Hold times for cleaned and disinfected areas and equipment
- Hold times for soiled areas and equipment
- Cleaning and disinfection performed after a shutdown or an excursion

Validation of Disinfectants

Table 23.0-1 Summary of EN Test Criteria for Registration for Established Claims

Organism Type	Test Method	Test Type	Contact Time (minutes)	Log Reduction Pass Criteria
Vegetative bacteria	EN 1276:1997	Suspension	5	5
Vegetative bacteria	EN 13697:2001	Surface	5	4
Vegetative fungi	EN 1650:1998	Suspension	15	4
Vegetative fungi	EN 13697:2001	Surface	15	3
Bacterial spores	EN 13704:2002	Suspension	60	3

*PDA Technical Report No. 70 Fundamentals of Cleaning and Disinfection Programs in Aseptic Manufacturing Facilities, 2015.

General Industry Efficacy Recommendations

- Suspension acceptance criteria
  - 4-5 log reduction

- Carrier/ Coupon acceptance criteria <1072>
  - 2 log reduction bacterial spores
  - 3 log reduction vegetative bacteria, yeast, mold spores

Disinfectants (PDA TR 70)

The classifications of sanitizers, disinfectants, and sporicides include the following:

- Alcohols
- Iodine/bromine-containing compounds
- Aldehydes
- Quaternary ammonium compounds
- Phenolic (EPA Data Call 2018 !)
- Hydrogen peroxide
- Chlorine and sodium hypochlorite
- Peracetic acid/hydrogen peroxide
- ß-Propiolactone
- Ethylene oxide
- Ozone
- Chlorine dioxide

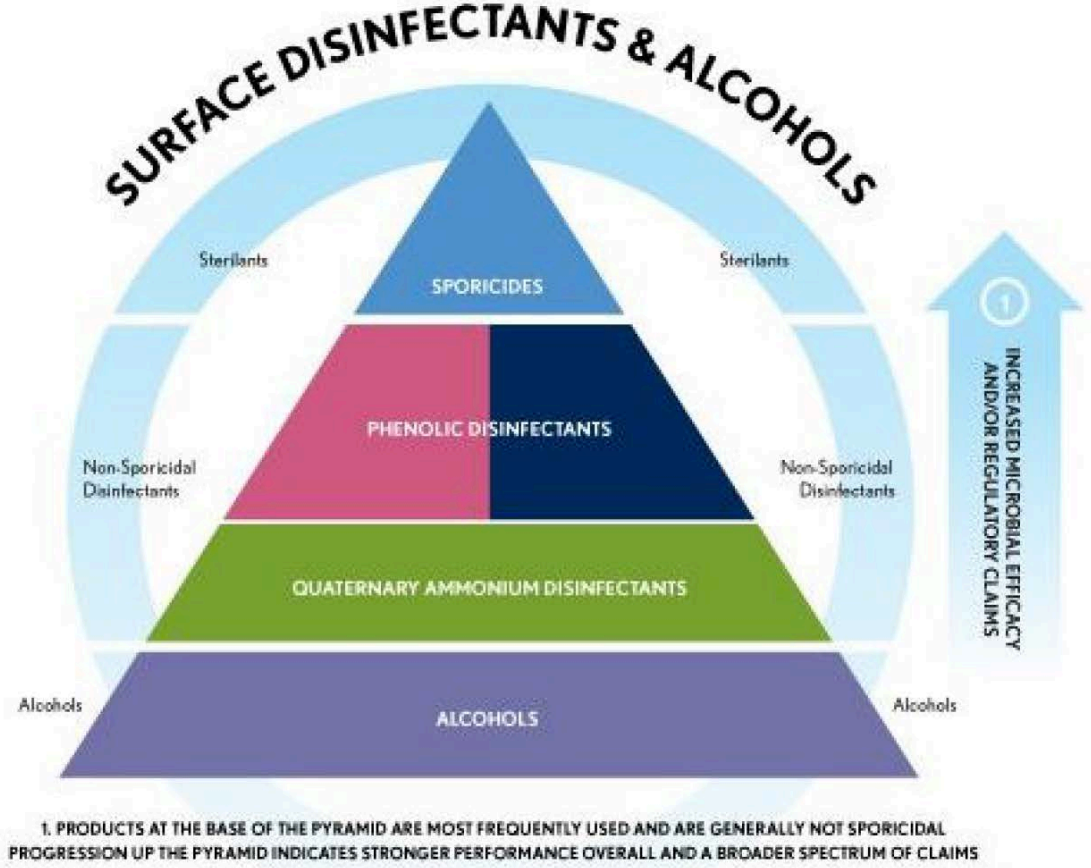
*PDA Technical Report No. 70 Fundamentals of Cleaning and Disinfection Programs in Aseptic Manufacturing Facilities, 2015.

Disinfectants

Disinfecting

- Isopropyl Alcohol or 70% IPA is one example
- Mode of action: denatures proteins, dissolves lipids and can lead to cell membrane disintegration
- Effectively kills bacteria but does not inactivate spores!
- Other sanitizing solutions
  - Chlorine
  - Lph st
  - Vesphene II

ISOPROPYL ALCOHOL (70%)



J. Polarine, 2016

C & D Steps (PDA TR 70)

For cleaning and disinfecting conducted on an established frequency in the Grade A and Grade B areas the following order is commonly followed (from lowest bioburden to highest bioburden) to ensure contamination from the cleaning process itself is minimized.

- A sterile cleaning agent (high surfactant based product) is applied to ceilings (not HEPA filters), then walls, then equipment is cleaned and finally the cleaning agent is applied to the floors in a succession from the furthest point to the closest point to the room exit. Mopping is the preferred method of application for ceilings, walls and floors.
- A squeegee is used to remove the excess liquid and contaminants from the ceiling (not HEPA filters), then walls and floors again in a succession from the furthest point to the closest point to the room exit.
- The dirtied liquid should be lifted from the area via a sterile dry mop, sterile dry wipe, or HEPA-filtered wet vacuum. This prepares the surface for the disinfecting agent.
- After the surfaces have dried they should be sufficiently wetted with a sterile disinfecting agent via mop, spray or wipe following the same sequence being used for the ceiling (not HEPA filters), walls, and floors as described above.

Spraying (PDA TR 70)

- **Spraying**

  This method produces the best wetting of surfaces. A spraying method that employs larger rather than smaller droplets has been found to provide better wetting results. As efficacy performance is based on saturation and penetration of the cell wall as well as contact time, this method produces very good results as long as the underlying surface has been appropriately cleaned. Spraying does not clean the surface, as it lacks mechanical action. Consistent spraying without routine use of a mechanical cleaning action will potentially result in the development of high residue levels, entrapped particulates, deteriorated surfaces, and, as the decontaminating agent will be unable to reach viable contaminants, increased bioburden levels.

Good wetting, but no cleaning

Mopping (PDA TR 70)

- **Mopping**

  Mopping assures that a mechanical action of cleaning is employed. The use of a mopping system for either walls or floors removes residues, viable contamination, and nonviable contamination. For walls, mopping is done from the highest surface point to the lowest surface point. For floors, mopping is done from cleanest to dirtiest and from the highest grade to the lowest grade. While mopping provides the mechanical action needed, great care must be taken to ensure surfaces are wetted appropriately. In general, mopping does not provide as uniform wetting as spraying. For example, the wringing of mop heads and the inability for mop heads to hold sufficient liquid may compromise the level of surface wetting and, therefore, the contact time required. As a result, while cleaning is accomplished, disinfection may be compromised.

Good cleaning, no uniform wetting

Wiping (PDA TR 70)

- **Wiping**

  Wiping with a presaturated cloth or a dry wipe that is wetted with a cleaning or disinfecting agent is a common practice in the cleaning industry. Wiping, as with mopping, cleans the surface of residues, viable contamination, and nonviable contamination with a mechanical action. Normally, wiping is associated more with cleaning than disinfection. Wiping is done on smaller surfaces that need to be cleaned, such as door handles, push plates, return vents, equipment, carts, and pass-through areas. While wiping possesses the ability to clean the surface, as with mopping, disinfection can be compromised as the surface wetting may not be sufficient to provide the required amount of disinfecting agent contact time. While wiping may remove viable contamination, great care must be taken to ensure that surfaces are adequately wetted.

For smaller surfaces, wetting may not be sufficient, ability to clean surfaces

Fogging or Gassing (PDA TR 70)

- **Fogging or Gassing**

This method can produce excellent results but does require longer periods of time to ensure adequate distribution of the agent and sufficient surface contact time. Fogging methods generate very fine droplets of the disinfecting agent, whereas gassing use a disinfecting agent in a gas form. While both are very effective, just as with spraying, they do not clean the surface. As a result, fogging or gassing without routine use of a mechanical cleaning action will potentially result in the development of high residue levels, entrapped particulates, deteriorated surfaces, and, as the decontaminating agent will be unable to reach viable contaminants, increased bioburden levels. Chemical agents that have commonly been used with this method of application are peracetic acid, hydrogen peroxide, phenol, bleach, quaternary ammonia, paraformaldehyde, and chlorine dioxide. Great care must be taken when a decision is made to use this method, as special safety considerations are required due to the potential exposure dangers and explosion hazards. See **Appendix VIII** for additional information on this method.

Fogging generates small droplets, or gassing : no cleaning

Resistance and Rotation (PDA TR 70)

This is also supported by the current USP <1072> Disinfectants and Antiseptics *(9)*:

> *The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance is less likely, as disinfectants are more powerful biocidal agents than antibiotics and are applied in high concentrations against low populations of microorganisms, so the selective pressure for the development of resistance is less profound.*

Based on this, the pharmaceutical and biotechnology industries have moved away from the rotation of two disinfecting agents. This formerly common practice led to high residue levels and subordinate efficacy performance. Today, most firms use a system whereby a disinfectant is rotated with a sporicide to more effectively reduce the bioburden levels. The rotation of a disinfectant with a sporicide is superior to the rotation of multiple disinfectants. If desired, the sole use of a sporicidal product that has proven efficacy can be implemented without a rotation. If used on a routine basis, the sporicide should destroy the level of contamination necessary to assure acceptable environmental conditions.

Rotation only with sporicides required

Categorization and Rotation of Disinfectants

- **Alcohols**: is also used for removal of other disinfectants/ 5- 10 min contact time (cell wall penetration)
- **Phenols**, **Quaternary Ammonium** compounds: broad range disinfectants-facility surfaces; non-corrosive
- **Chlorine; Hydrogen peroxide** (also combined with Peracetic Acid): are effective sporicidal agents

Rotation: sporicidals used on a limited basis (e.g. weekly or monthly)

Cleaning and Disinfection

- Cleaning is an important prerequisite to disinfection.
- If the surfaces are not clean there is a greater risk that the disinfection process may be ineffective.
- This can be cause by:
  - Soiling material (dirt) physically preventing the disinfectant from coming into contact with any underlying organisms
  - Soiling material reacting with the disinfectant and inactivating it.

Cleaning and Disinfection of Non-product Contact Surfaces and Work Surfaces

- 1. Precleaning (if required)

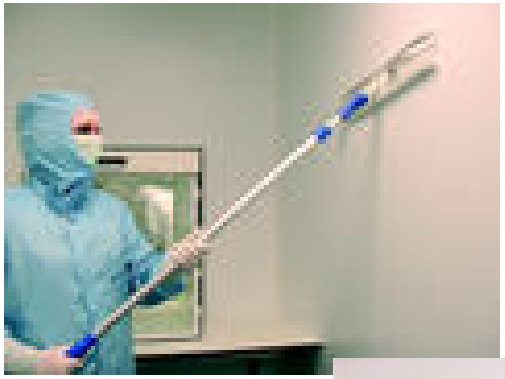
- 2. Disinfection: disinfectant or sporicide (spraying with a 3- 5 min contact time)

- 3. Dry wipe down or 70 % spray down - followed by a dry wipe in case of residue from disinfection step.

OR use: Combination of Cleaning & Disinfectants

Disinfection- Practices

- Floor: Mopping from the cleanest to dirtiest
- Walls: Mopping from highest to lowest surface point
- 3 or 2 buckets / single use mops/ wipes
- Cleanrooms: From higher to lower grade



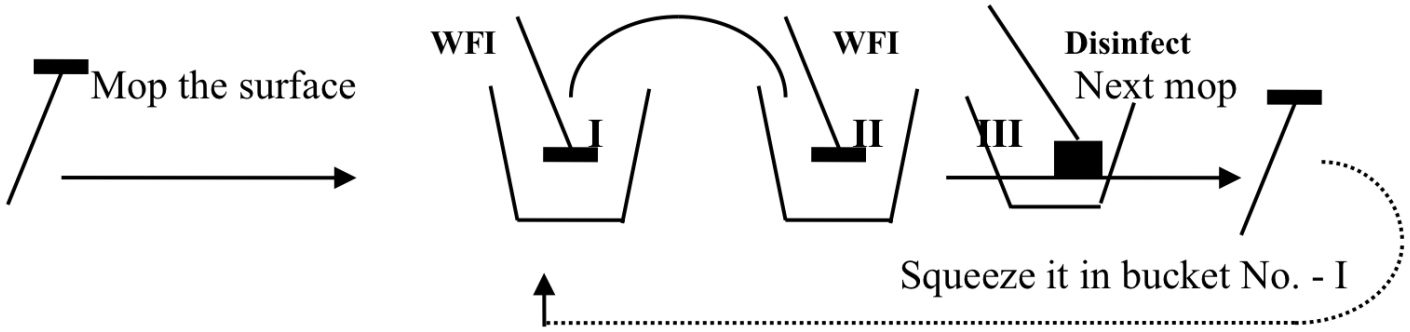
TR 70

- Three-bucket system
  - Bucket 1 and bucket 2 both contain the disinfectant (based on SOP). Bucket 3 is the wringing bucket and starts out empty.
  - The mop is first placed into the rinse bucket (bucket #2), wring out in bucket #3, and place in sanitizing bucket (bucket #1), wring out in bucket #3 and apply to the surface. After each pass, replace the mop into the rinse bucket, rinse and wring, and place mop into bucket #1 – sanitizing bucket, wring. Repeat the steps.

Three Bucket Method

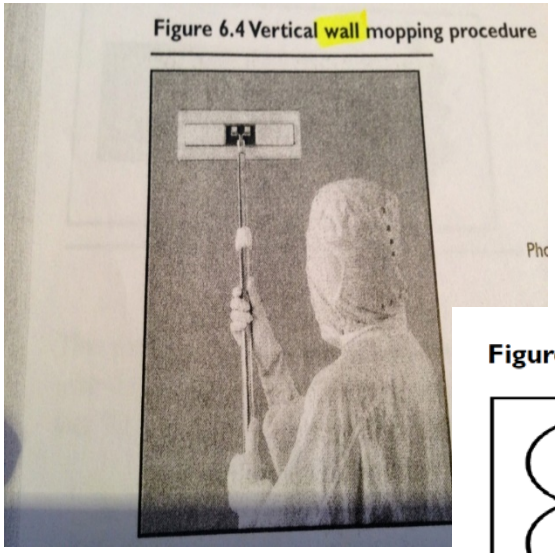
Three-bucket system

Three bucket as shown below should be used; One, for collecting the squeezed dirty water. Second, one dipping of the mop into clean Water for injection and the third one with disinfectant solution.



Pull-lift method

- Floor and Walls: Total 3 strokes overlapping; each less than four feet in length, mop pulled toward the operator.



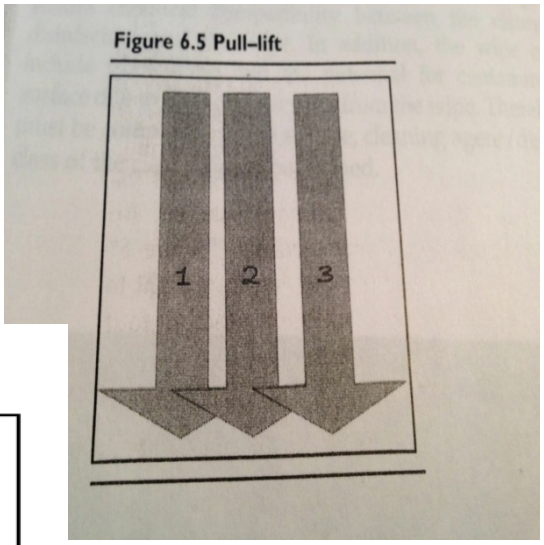
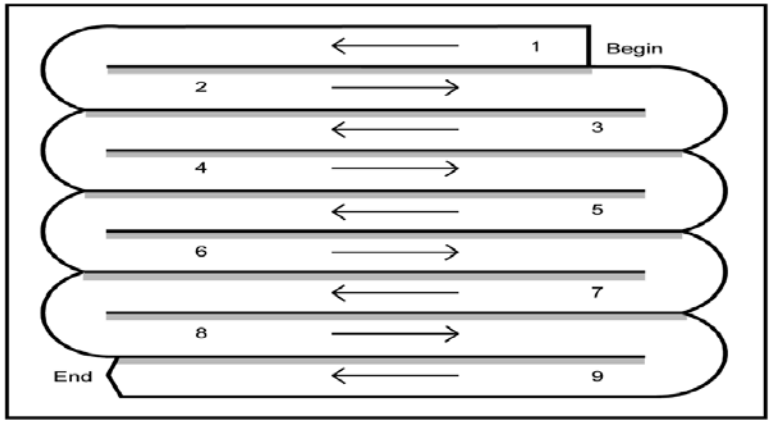


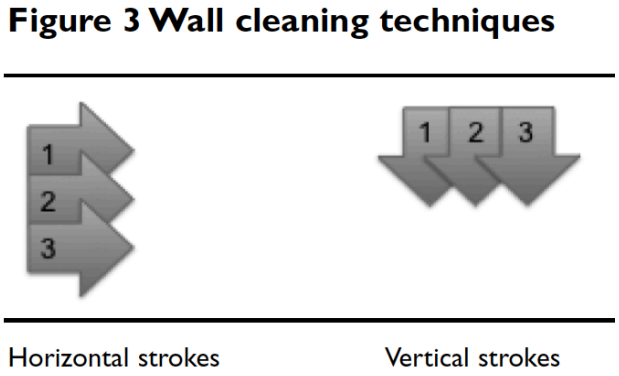
Figure 4 Modified figure "8"



Source: Anne Dixon- H.

Horizontal Mopping

- Is used for work stations
  - Source: Anne Dixon- H.



- Requires complete coverage with a film to allow contact times at validated concentrations
- Contact time is generally 5- 10 min

Prevent residues of disinfectants !

Important Points I

- Validate your disinfectants (Carrier test) with all materials from your cleanrooms
- Use “sterile“ disinfectants within grade self- A/ B, and perform periodic microbial count testing of your prepared disinfectants (in their final container – including sprayheads)
- Label the disinfectants flasks with defined expiry dates
- Aseptic Practices:
  - For gloves use dispensers, not spray- bottles
  - Prevent bottle shuttle between zones A and B

Important Points to Remember II

- Cleaning Practices:
  - Use combination- disinfectants (cleaning/ disinfection)
  - If clean, limited usage of detergents
  - Additional (sterile) single use IPA wipe may be required
- Use at least a 2 Bucket methods or systems with single use , disposable mops and wipes
- Follow a correct sequence
- Establish detailed cleaning/ disinfection programs – in SOP & very detailed Checklists (pictures should be included)
- Have a very detailed documentation about the Cleaning and Disinfection activities in a logbook.

Important Points III

- Within Grade A :
  - Sterile IPA towels usage (during AseptOps); and after filling (of cabinet)
  - No spraying during operations
- Filling cabinet - Environment:
  - sporicidial (at least weekly)
- Sporicidial Disinfection: Corrosion risk
- (Direct/ Indirect) Product Contact Surfaces- NEVER Disinfect !
- Finally: Intensively Train your Clean Personnel (and include them in the Environmental Monitoring Program)

Gowning Procedures & Qualification

Important points to consider

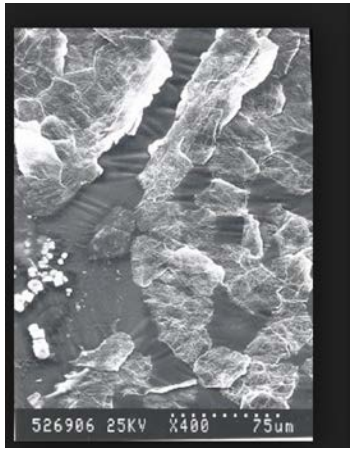
- Handwashing
- Glove Wearing
- Disinfection
- What may go wrong ?

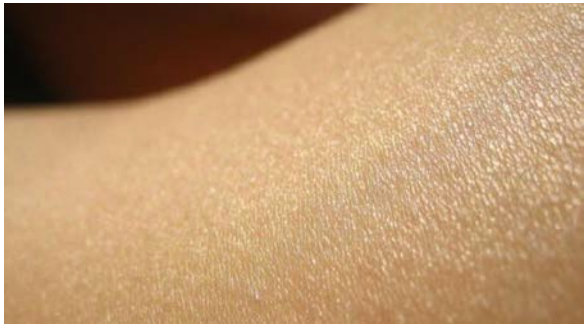
Skin flakes !!!

Of those billions of skin cells, between 30,000 and 40,000 of them fall off every hour. Over a 24-hour period, you lose almost a million skin cells [source: Boston Globe].

In one year, you'll shed more than 8 pounds (3.6 kilograms) of dead skin.
10 g per day !

Some cells, like skin cells, are constantly dividing. We need to continuously make new skin cells to replace the skin cells we lose.





The majority of contamination in a clean room comes from people

- While sitting motionless, a person sheds about 100,000 particles
- While walking at 8 Km/H, a person can shed up to 10 million particles **PER MINUTE!**

EU Annex 1 (2020)- Draft

i. Grade A / B: Dedicated garments to be worn under a sterilized suit. Sterile headgear should enclose all hair (including facial hair) and where separate from the rest of the gown, it should be tucked into the neck of the sterile suit. A sterile face mask and sterile eye coverings (e.g. goggles) should be worn to cover and enclose all facial skin and prevent the shedding of droplets and particulates. Appropriate sterilized, non-powdered, rubber or plastic gloves and sterilized footwear (such as overboots) should be worn. Trouser-legs should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should minimize shedding of fibres or particulate matter and retain particulates shed by the body. Garments should be packed and folded in such a way as to allow operators to gown without contacting the outer surface of the garment.

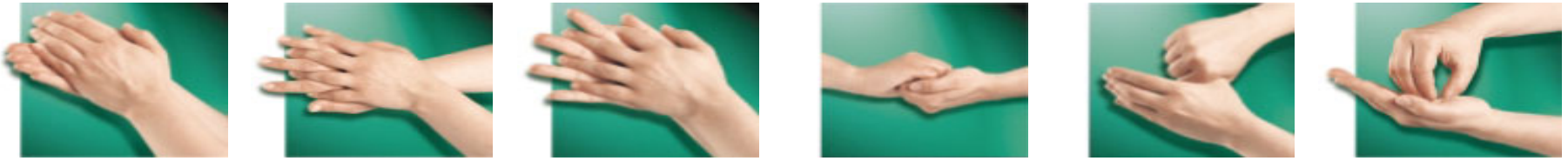
ii. Grade C: Hair, beards and moustaches should be covered. A single or two-piece trouser suit gathered at the wrists and with high neck and appropriately disinfected shoes or overshoes should be worn. They should minimize the shedding of fibres and particulate matter.

iii. Grade D: Hair, beards and moustaches should be covered. A general protective suit and appropriately disinfected shoes or overshoes should be worn. Appropriate measures should be taken to avoid any ingress of contaminants from outside the clean area.

iv. Gloves should be worn in Grade C and D areas when performing activities considered to be a contamination risk as defined by the CCS.

Hand Washing / Hand & Gloves Disinfection

- **Correct Disinfection of Hands and Gloves**
- **Correct Exposure Time**



Hand wash and Glove wearing video

Video

Disinfect hands and wear Gloves/correct Gowning

Video

Put on First Pair of Gloves

Put on First Pair of Gloves (continued)

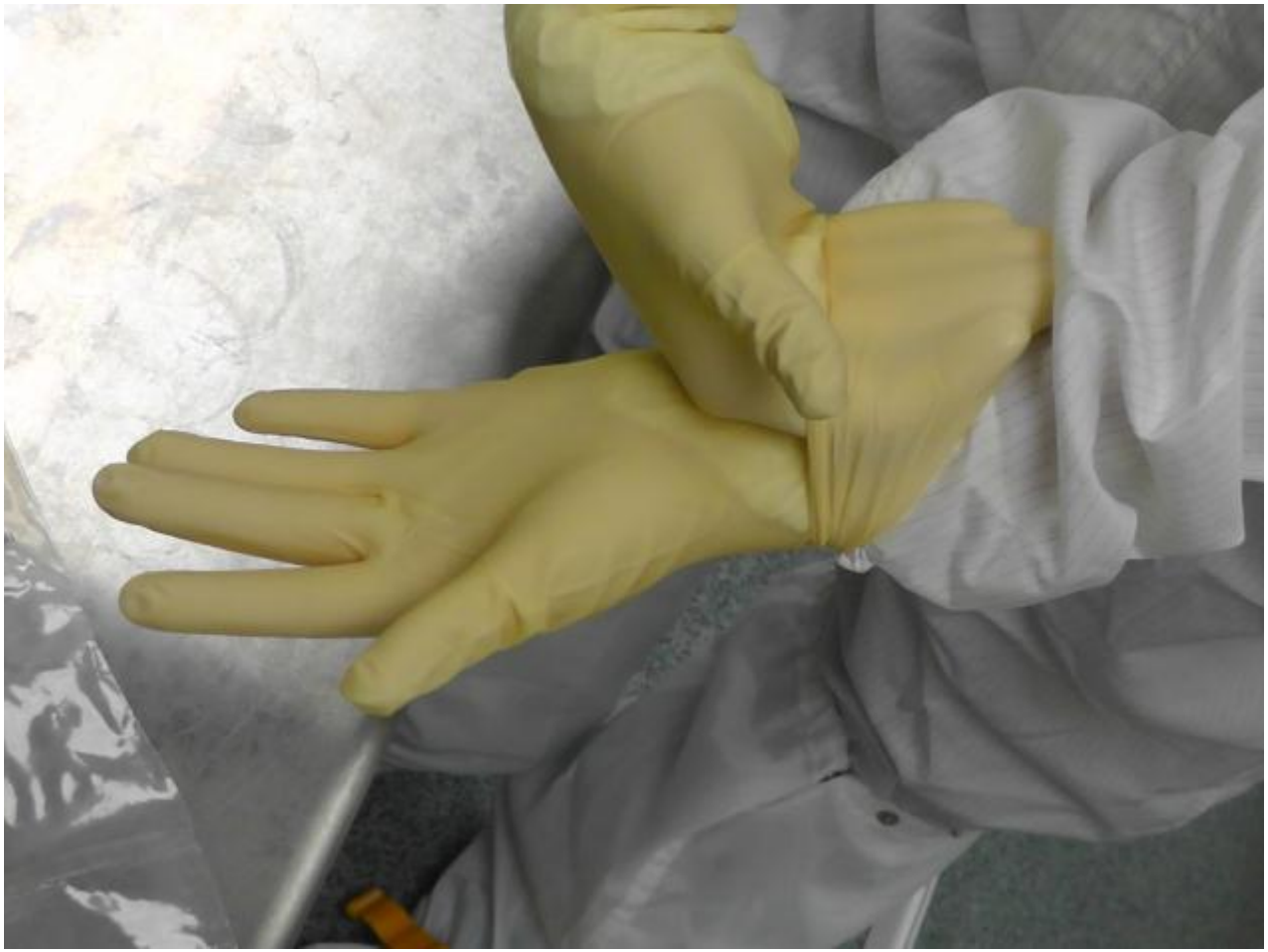
How to perform ?

Mask

Coveralls

Step Over Bench

Second Pair of Gloves

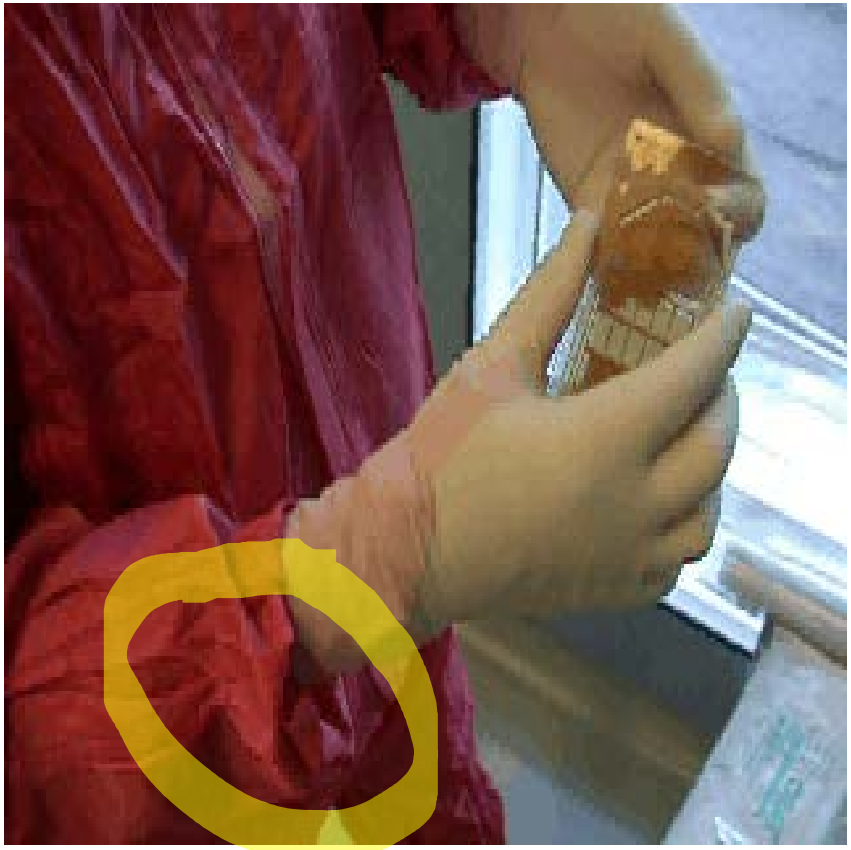


Ready for Work

Gowning

- No skin is exposed once gowning is complete

Longer gloves (2 gloves)



What´s wrong here ?

Gowning Qualification

What is important :

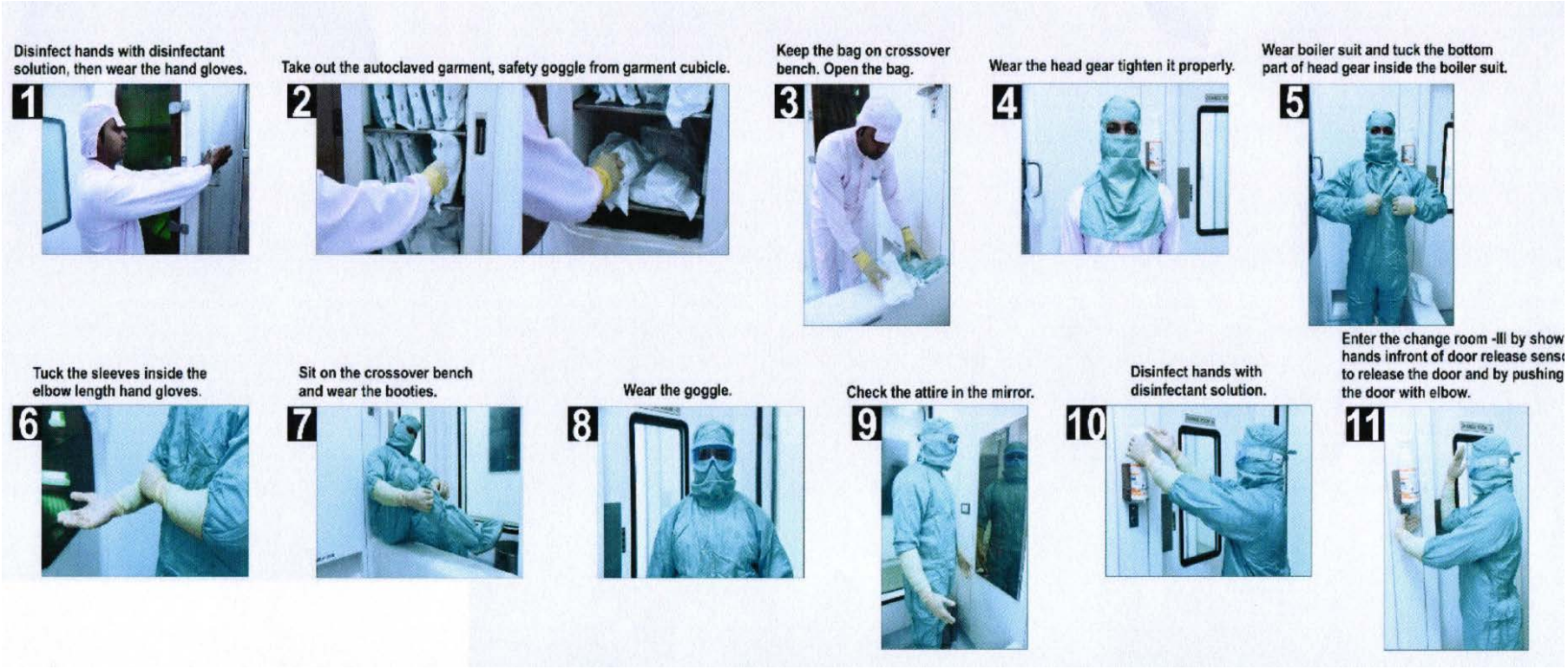
- Practical Training in classroom before qualification
- QA oversight is required
- Visual review of practical performance
- Surface Monitoring of gowning and gloves
- Typically 3 times
- Certificate for entering cleanrooms

Gowning Practices

Important points to consider

- What might go wrong ?
  - Space limitations/ no separate INLET/ EXIT
  - Wrong gowning and cleanroom concept
  - No boots after bench to step in
  - Gowning touches floor (except integrated shoes)
  - Handwashing and Disinfection procedures
  - Too short second pair of gloves
  - No mirrors and no pictures / descriptions
  - 3 layers of gowning / RH and T/ Goggles fogging/ ..
  - Control of number of washing cycles / supplier

Good Example about gowning description



3 Take Away Messages :

- Prevent too complicated gowing procedures
- Visual aids e.g. videos are very supportive for training
- Do stretching before entering grade B