Sterilization Processes – 2024

What do we do now?

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EMA / Annex 1 – Sterilisation
FDA / USP – Sterilization

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A Growing and Unfortunate Divide

USA

- FDA – guidelines are flexible with exceptions rather common.
- Multiple layers of internal review & field inspection.
- USP – extensive sterilization content (50+ pages) that is relatively current (2012-2019). Includes all major sterilization methods.
- Many guidelines with general requirements with few specifics.
- Interpretive – What to do!

EU

- EMA – guidelines are more restrictive with fewer exceptions.
- Reviewer and filed inspector more often the same person.
- EP – limited sterilization content (4-5 pages) from 2008. Limited to steam, dry heat, radiation and ETO.
- Annex 1 – includes many specific requirements restricting flexibility of execution.
- Substantive – How to do!

USP *Sterilization* <1229. -01 - 12

- 1229.00 – Introduction & General Principles
- 1229.01 – Steam Sterilization by Direct Contact
- 1229.02 – Moist Heat Sterilization of Aqueous Liquids
- 1229.03 – Monitoring of Bioburden
- 1229.04 – Sterilizing Filtration of Liquids
- 1229.05 – Biological Indicators for Sterilization
- 1229.06 – Liquid Phase Sterilization
- 1229.07 – Gaseous Sterilization
- 1229.08 – Dry Heat Sterilization
- 1229.09 – Physicochemical Integrators and Indicators for Sterilization
- 1229.10 – Radiation Sterilization
- 1229.11 – Vapor Phase Sterilization
- 1229.12 – New Sterilization Methods

USP *Sterilization* <1229. -13 - 20

- 1229.13 – Sterilization-in-Place
- 1229.14 – Sterilization Cycle Development
- 1229.15 – Sterilizing Filtration of Gases
- 1229.16 – Prion Inactivation
- 1229.17 – Mycoplasma Sterilization
- 1229.18 – Viral Clearance
- *1229.19 – Simultaneous Decontamination & Sterilization*
- *1229.20 – Sterilization: Pre-, Mid-, and Post-Process Considerations*

Related Chapters

- 55 - Biological Indicators – Resistance Performance Tests – *Revised*
- 56 - Methods for Determination of Resistance of Microorganisms to Sterilization Processes – *all new content*
- 1211 – Sterilization & Sterility Assurance – *all sterilization process related content deleted, substantial number of other changes*

Annex 1 - Sterilization / Sterilisation

- The content in this section is heavily influenced by its Orange Guide origins; HTM10, EN285 and pretty much anything written on the subject in the UK!
- US sterilization practices differ markedly. Which practices will your investigator / auditor / reviewer accept? Harmonization on which set of expectations?
- Understand that the *European Pharmacopiea* includes only steam, dry heat, gas (ETO) or gamma irradiation.
- Compare this to USP which includes moist heat, dry heat, gas (ETO, O_3, ClO_2, NO_2), liquids, radiation (gamma, X-ray, E-beam), and vapors (H_2O_2, peracetic acid).

Sterilization / Decontamination with H_2O_2

- In 2018, an MHRA blog post stated, *"… our current stance is that VHP cannot be used to sterilise critical items."*
- The concern was raised regarding the reports of 'rogue' BI results suggesting the vapor process is inherently fragile.
- This has curtailed H_2O_2 use in various applications with isolator decontamination / sterilization the most impacted.
- The problem may be due to false beliefs, inadequate system design, BI configuration and more.
- **FDA has approved deep vacuum H_2O_2 sterilization processes which are not the same.**

Sterilization of Isolator Installed Equipment

- *"Particular attention should be given when the adopted product sterilisation method is not described in the current edition of the Pharmacopoeia, …"* [8.37]

- Vapor sterilization for RABS & isolators where *"e.g. sterilised items such as stopper bowls and guides, and sterilised components"* are sterilized would be non-compliant. [5.5]

- This is a major challenge which cannot be easily addressed. It increases manual activity and contamination risk as well.

Vapor Sterilization / Decontamination Practices

Troubles and Traumas

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Process Difficulties due to ‘Rogue’ BI’s

- Positive BI results in sterilization / decontamination systems have caused difficulties in initial / periodic validation studies.
- Project delays and supply interruptions have resulted.
- The MHRA blog 2018 suggested imposing a ban on VPHP for the sterilization of product contact surfaces. EMA Annex 1 2024 is aligned with that belief. FDA is characteristically silent.
- Unknown variations in BI’s are suspected to be ‘root cause’.

- Do ‘rogues’ exist and if so, can they be eliminated?

Vapor Processes & Thermodynamics

- All sterilization process seek uniform conditions throughout.
- Vapor processes also tend towards equilibrium, but this leads to condensation and the inevitable presence of two phases.
- This results in non-uniformity in phase and concentration and inconsistent lethality across the system.

- Aggressive mixing could reduce variation, but current designs are rarely sufficient.
- Most two-phase vapor systems have insufficient mixing, and some rely totally on unidirectional air, which is the exact opposite of what is needed to create uniformity.

Gases, Liquids & Vapors

Gas

Liquid

Vapor

Cloud

Mist

Fog

Rain

Physical-Chemical Reality of VPHP Processes

- The process may not reach equilibrium due to continued heat input during process and temperature variations (inlet to exhaust) across system.
- Temperature varies with time and location, and so will phase and concentrations. The amount of condensed H_2O_2 and H_2O varies with local temperature.
- Precise determination of conditions across the entire system and its surfaces is impossible.

BI Differences are Problematic

Glassine Envelope

Paper Strip w/ Gst Spores

Gases

Liquids

Gases & liquids can penetrate both envelope & paper

Tyvek® Envelope

SS Coupon

Gst Spores on surface

Gases

Liquids

Gases penetrate Tyvek, but liquids do not.
Nothing can penetrate stainless steel.

‘Dry’ vs ‘Wet’

Dry – avoid condensation

- Vapors are gases, it’s always a single-phase
- **Gas H_2O_2 kills quicker than liquid H_2O_2**
- Avoid condensation
- Mixing is not a concern
- Less H_2O_2 used

Wet – encourage condensation

- Vapors are 2-phase mixtures of gases and liquids
- **Liquid H_2O_2 kills quicker than gas H_2O_2**
- Condensation is desirable
- Mixing for greater uniformity
- More H_2O_2 used

It doesn’t matter what we try to do, both phases will always be present

Slow Kill Death Curve

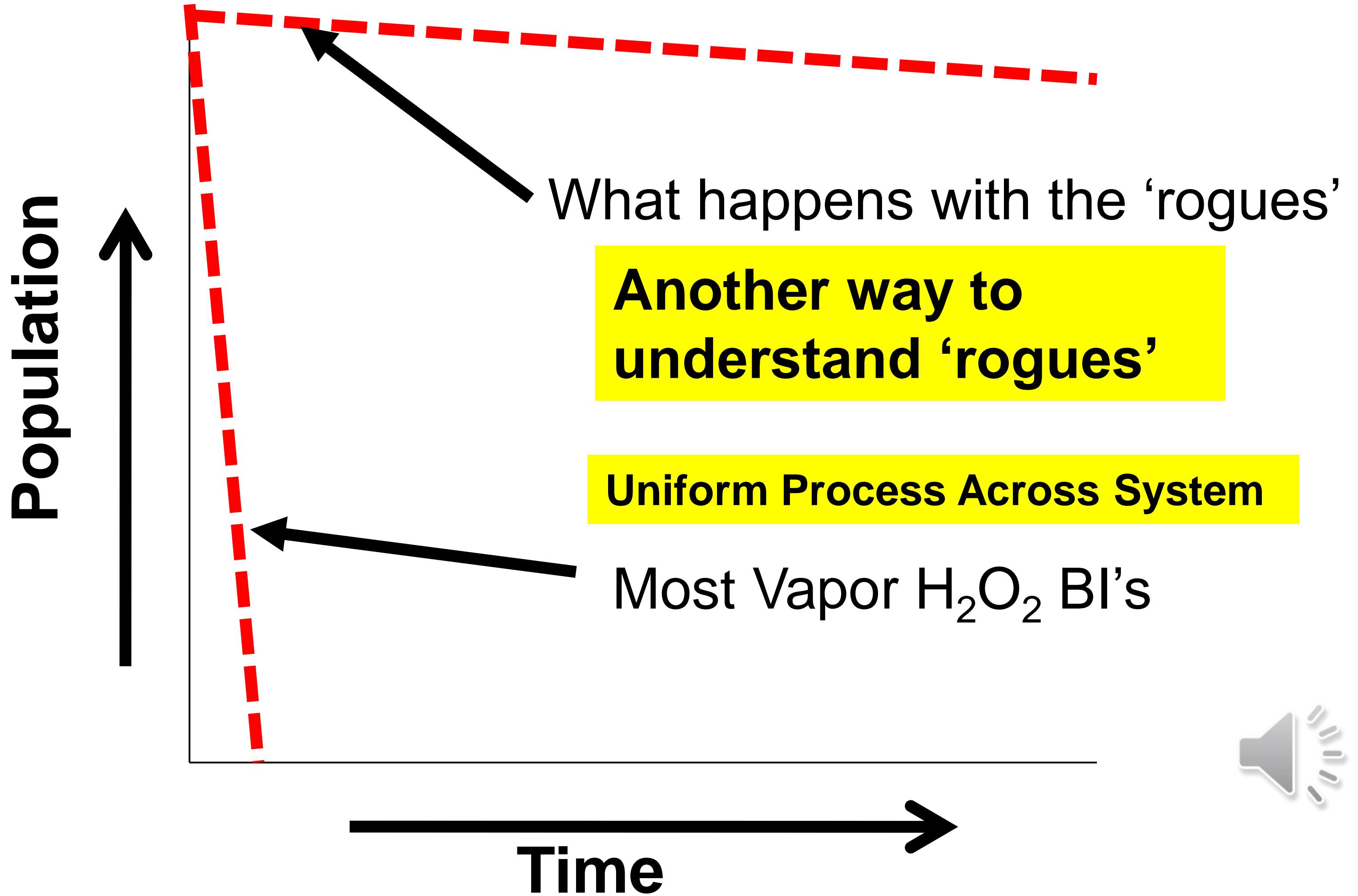
The death curves aren’t really straight.

Uniform Process Across System

Fast Kill Death Curve

Population

Time



What happens with the 'rogues'
Another way to understand 'rogues'
Uniform Process Across System
Most Vapor H_2O_2 BI's
Population
Time

Number of BI's in Study

Most vapor H_2O_2 BI's respond like this

Uniform Process Across System

But it only requires a few 'rogues' to create havoc

Time

Another Way to Look at Vapor Processes

- It’s understood that process conditions directly impact process lethality.
- For example, in thermal sterilization a higher process temperature results in a lower D-value or a steeper death curve. This is true for all processes, the more aggressive the conditions the easier it is to kill microorganisms.
- If the conditions are non-uniform, then there’s the potential for different lethal effects in different places.
- Let’s apply that thinking to H_2O_2 vapor processes.

Death curve where conditions are inadequate

It doesn't matter which phase is which

All BI's with the same resistance

Death curve where conditions are optimal

Population

Time

Most locations deliver high lethality

All BI's with the same resistance

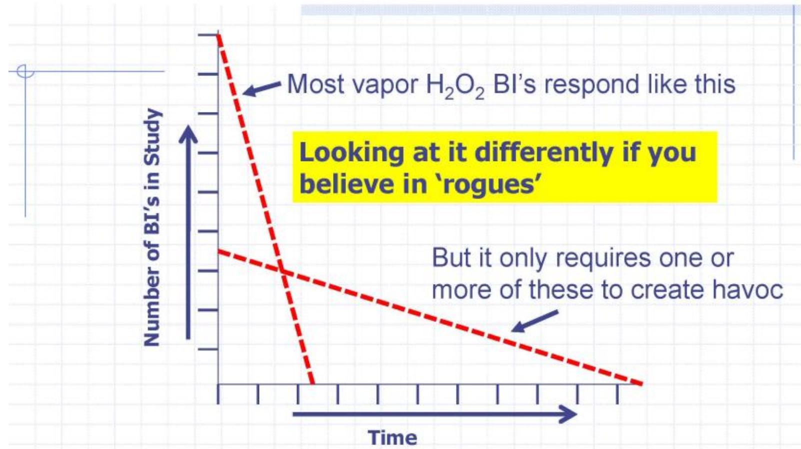
But having locations like this causes problems

Number of BI Locations

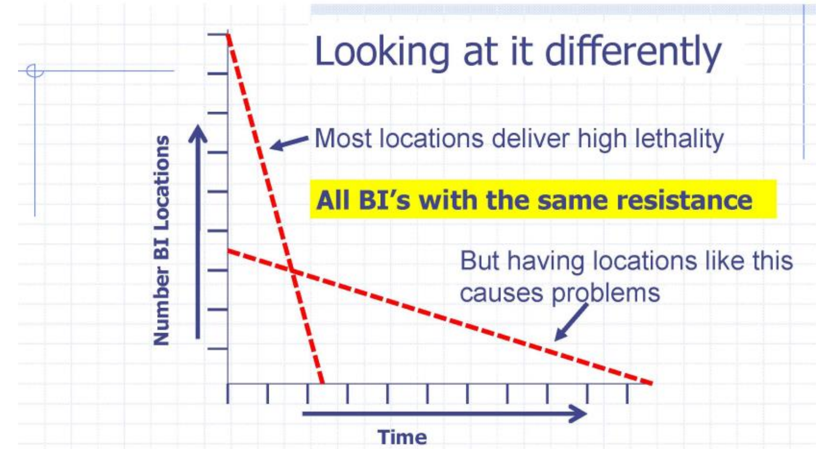
Time

What’s the Real Root Cause of Positive Results?

It’s a ‘Rogue’



It’s a Non-Uniform Process



The similarity in appearance it not a coincidence, it serves to inform that there’s no reason to believe that ‘rogue’ BIs are the only cause of anomalous results.

The Unfortunate Reality

- BI configurations are often sub-optimal.
- The unavoidable variations in vapor H_2O_2 processes may cause anomalous results unrelated to the biological indicator but due to varying conditions across the system.
- The complexities of the vapor process can give the appearance of ‘rogues’ even where every BI is identical and free of defects.
- The kill rate curves shown are simplified. There’s an infinity of them between the extremes and kill rates at any location will change during the process.
- **The death rate curves are not straight lines.**

Use the most suitable Biological Indicators

- Pick a reliable supplier with sufficient experience.
- Avoid use of planar stainless-steel coupons, use non-cellulose fiber strips as the BI substrate.
- Use a non-Tyvek protective wrap or remove the wrap before exposure (false positive results with *G. stearothermophilus* spores are nearly impossible).
- Don’t bother with multiple BI’s in locations, its preferable to use the same total number of BI’s in more locations.
- Use a challenge level of 10^4 spores/strip unless sterilization is required, use 10^6 spores/strip for those items and surfaces.

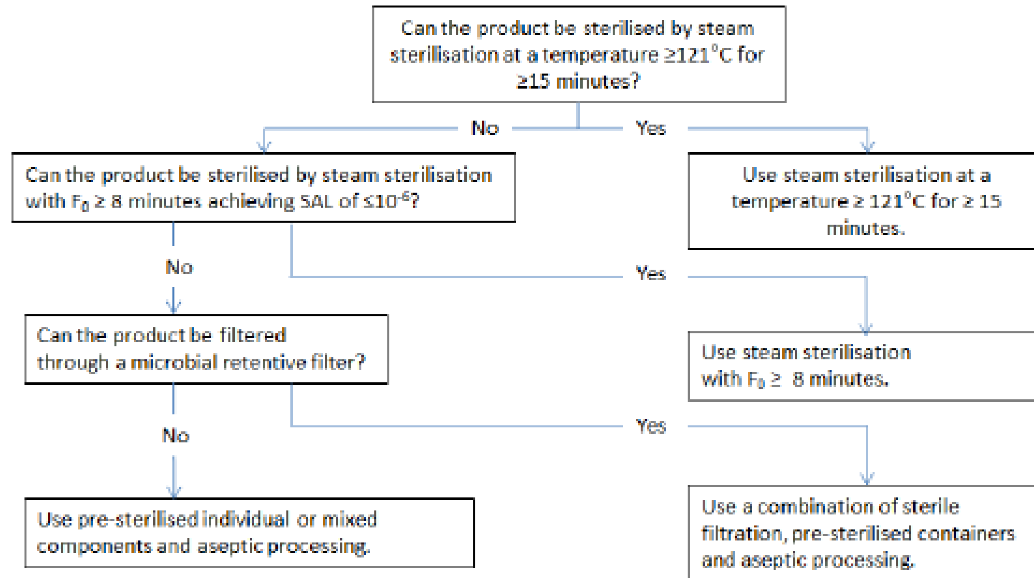
Use the most lethal process you can

- Forget the idea that condensate is to be avoided, that's likely wrong. Use enough H_2O_2; but not so much as to have drops form on surfaces.
- Dehumidification which removes H_2O is not beneficial, it will not alter the amount of H_2O_2 that will condense.
- Use more internal mixing, unidirectional air works against uniformity and should not be used. This works to advantage in aeration as well. The more mixing the better.
- Aeration times may not change much as micro-condensation has always been present, just not always enough to effect consistent kill at all locations.

Annex 1 / FDA
Terminal Sterilization

Annex 1 Terminal Sterilization

- The Annex 1 included content is non-controversial.
- The elephant in the room is EMA's Guideline on the sterilisation of the medicinal product, active substance, excipient and final container. EMA\CHMP\CVMP\QWP\850374\2015



Sterilization – USP <1229>

- *"Articles intended to be sterile must attain a ≤10^{-6} probability of a non-sterile unit, i.e., less than than or equal to 1 chance in 1 million that* ***viable bioburden*** *microorganisms are present."*
- What does this mean in the real world?
  - **The process needs to reliably destroy the bioburden.**
  - **No minimum F_0 requirement.**
  - **No minimum time or temperature.**
  - **No specified BI population or kill.**
  - **No defined sterilization process.**
- It allows for flexibility in sterilization process design to achieve the required result free of arbitrary requirements.

Calculation of PNSU / SAL

$$\log N_u = \frac{-F}{D} + \log N_0$$

where:

N_u	**=**	**PNSU / SAL**
D	**=**	**D-value of the natural bioburden**
F	**=**	**F-value (lethality) of the process**
N_0	**=**	**bioburden population**

The Terminal Sterilization Dilemma

- The goal of sterilization must be balanced against the need to maintain the product's essential quality attributes.

Microorganisms and Growth

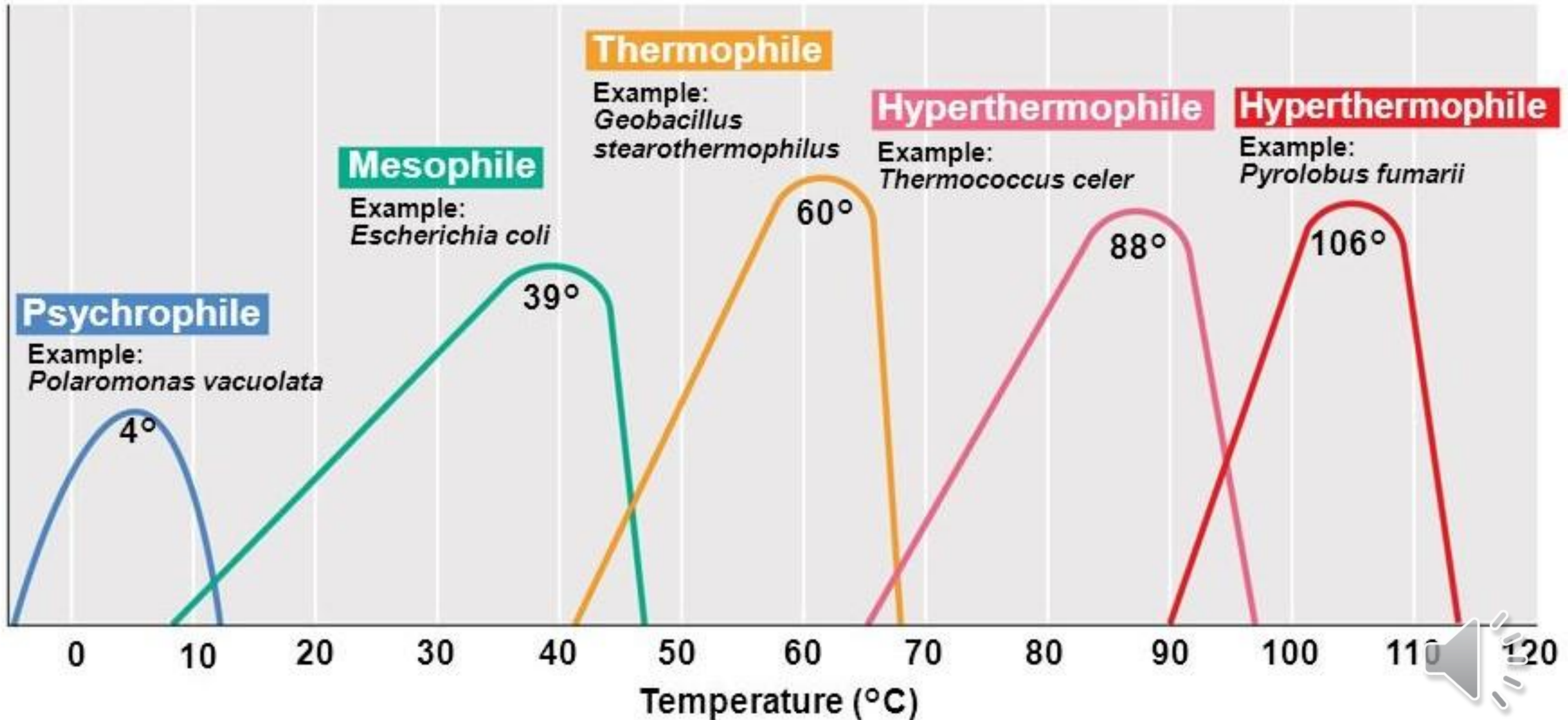


Table 1 – Calculated PNSU for selected pathogens at 80°C / 20 min & 100°C for 5 minutes*

Microorganism	D_{60} – Pflug reported (minutes)	z-value Pflug reported (°C)	D_{80} – estimated** (minutes)	D_{100} – estimated** (minutes)	Assumed N_0**** (CFU)	Estimated PNSU @ 80°/20 minutes***	Estimated PNSU @ 100°C / 5 minutes***
Aeromonas hydrophilia	0.066	6.0	0.00007	0.00000007	100	$10^{-285,000}$	$10^{-71,400,000}$
Campylobacter jejuni	0.35	6.5	0.0004	0.0000004	100	$10^{-50,000}$	$10^{-12,500,000}$
Escherichia coli	2.0	5.0	0.0002	0.00000002	100	$10^{-100,000}$	$10^{-250,000,000}$
Listeria monocytogenes	16.7	6.5	0.02	0.00002	100	$10^{-1,000}$	$10^{-50,000}$
Salmonella species	2.5	5.6	0.003	0.000003	100	$10^{-6,600}$	$10^{-1,666,000}$
Staphylococcus aureus	3.0	9.5	0.03	0.0003	100	10^{-600}	$10^{-16,600}$
Vibrio species	2.6	6.0	0.003	0.000003	100	$10^{-6,600}$	$10^{-1,666,000}$
Yersinia enterocolitica	5.0	5.0	0.0005	0.00000005	100	$10^{-40,000}$	$10^{-100,000,000}$

* D_{60} and z-value data in this table from Pflug.[18]

** D_{80}, and D_{100} values calculated from Pflug and rounded off for ease of calculation.

*** PNSU values rounded off for ease of comprehension

**** Note that the N_0 values have no meaningful impact on the estimated PNSU.

Some Successful Terminal Sterilization Projects

High temperature / Short Time Syringe 2017

Overkill TS process

F_0 >15 TS process

F_0 8-10 TS process 1985

F_0 2-4 process 2004

~100°C process 2015

~80°C process

~60°C process

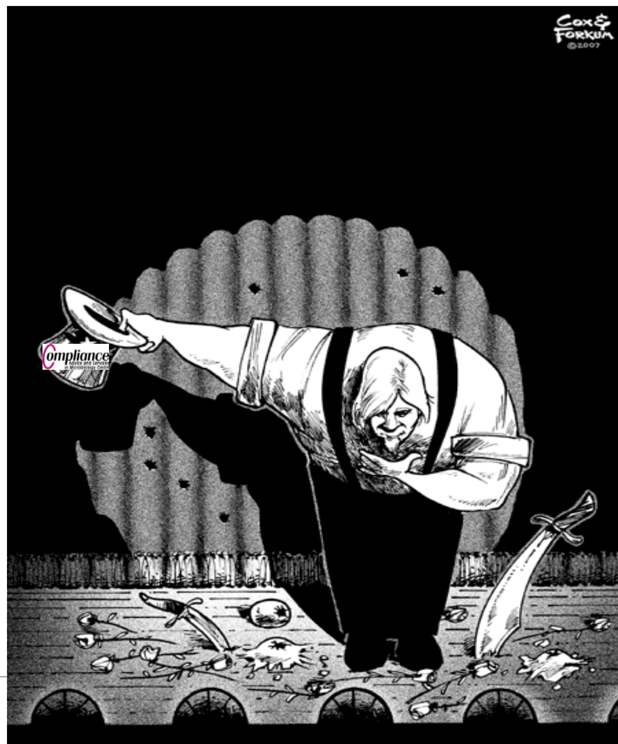
Not yet, but following an AP they would increase patient safety. I need a client brave enough to try.

Aseptic Fill

Biological Impact

Estimated Physical Lethality

Thanks for Your Attention!



Questions?

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