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Validation of Sterility Assurance Level Up To 10^6 Log Reductions

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Abstract: An attempt was made to validate Sterility Assurance level (SAL) of two Industrial Autoclaves up to 10^6 Log Reduction by using both gram negative and gram positive bacteria. For gram negative bacteria, a suspension of *E. coli* was prepared at 10^8 cfu/ml which was sterilized individually through two autoclaves at 121^0C for 20 minutes at three consecutive cycles. Bacterial load was then determined and found 8 log reductions of bacterial growth. For Gram positive bacteria, 12 ampoules of *Geobacillus stearothermophilus* containing 10^6 cfu were placed in two Autoclaves. After sterilization, no growth of *Geobacillus stearothermophilus* was found which also proving the 10^6 Log Reductions of that sterilization parameters.

Keywords: SAL, Log Reduction, Sterilization, *Geobacillus stearothermophilus*, Autoclave.

INTRODUCTION

Sterility assurance level (SAL) is the probability of a single unit being non-sterile after it has been subjected to sterilization. In microbiology it is impossible to prove that all organisms have been destroyed as the likelihood of survival of an individual microorganism is never zero, so SAL is used to express the probability of the survival. SALs can be used to describe the microbial population that was destroyed by the sterilization process. Each log reduction (10^{-1}) represents a 90% reduction in microbial population.

A process that achieves a "6-log reduction" (10^{-6}) will theoretically reduce an initial population of one million organisms to very close to zero. Use of Autoclave is common to provide the greatest assurance of sterility for critical products such as implantable devices or injectable drugs [1, 2]. An autoclave is a pressure chamber used to carry out industrial processes requiring elevated temperature and pressure different from ambient air pressure to sterilize medical or laboratory instruments by heating them above boiling point [3]. To assess the effectiveness of any sterilization process, scientists use a unit of measure called sterility assurance level, or SAL [4]. SAL is a result of sterilization and is normally intended to imply a certain degree of microbial inactivation imparted by a sterilization process using heat [5]. In our study, we tried to reveal the fact that whether the Autoclave, using moist heat is capable of ensuring sterility up to 6-log reduction" (10^{-6}) level or not.

METHODS

For this study, two Laboratory Autoclaves was used.

- Astell SR 100 (320 Litre)
- Unisteri SL SPSL636-2FD (160 Litre)

For gram negative bacteria Lyophilized *E. coli* pellet (ATCC 8739) was used. For preparing suspension of E. coli up to 10^8, it was compared with 0.5 McFarland Solution [6]. Microorganisms load was then determined by serial dilution and Pour Plate Method [7]. 10 ml 10^8 *E. coli* suspension of three test tubes was then placed in Autoclave with liquid load and ran in three individual liquid cycles by moist heat sterilization of two Autoclaves. Autoclave cycle was run at 121^0C and 15 PSI for 20 minutes. After sterilization, microbial load was again determined by serial dilution and Pour Plate Method [7]. For Gram positive bacteria, *Geobacillus stearothermophilus* ampoules from NAMSA (NAMSA Code: SCS-06) which containing 10^6 population was used. 12 Ampoules were used for each individual solid cycle and placed in different position of loaded Autoclave Chamber. Three cycle of each Autoclave was run. After that, the ampoules were incubated at 60^0C for seven days with a positive

control. Log reduction was then calculated by comparing with initial population.

RESULTS AND DISCUSSIONS

Three suspension of *E. coli* for each individual liquid cycle was prepared at 10^8 cfu/ml. Initial count of all Suspension (Table: 1) was recorded. These suspension was ran in six individual liquid cycles by moist heat sterilization of two Autoclave and Log reduction was calculated by comparing with initial count (Table 2).

Table-1: Initial count of suspension

Suspension description	Count of three test tube		
	Test Tube 1	Test Tube 2	Test Tube 3
Suspension for Astell Autoclave cycle 1	4×10^8	2×10^8	2×10^8
Suspension for Astell Autoclave cycle 2	1×10^8	1×10^8	9×10^8
Suspension for Astell Autoclave cycle 3	9×10^8	5×10^8	7×10^8
Suspension for Unisteri SL Autoclave cycle 1	3×10^8	2×10^8	5×10^8
Suspension for Unisteri SL Autoclave cycle 2	8×10^8	7×10^8	6×10^8
Suspension for Unisteri SL Autoclave cycle 3	1×10^8	4×10^8	9×10^8

Table-2: Log reduction from initial count

Suspension description	Count of three test tube		
	Initial Count	Count after sterilization	Log Reductiom
Suspension for Astell Autoclave cycle 1	4×10^8	<10	8.64
	2×10^8	<10	8.35
	2×10^8	<10	8.35
Suspension for Astell Autoclave cycle 2	1×10^8	<10	8.05
	1×10^8	<10	8.05
	9×10^8	<10	9
Suspension for Astell Autoclave cycle 3	9×10^8	<10	9
	5×10^8	<10	8.74
	7×10^8	<10	8.89
Suspension for Unisteri SL Autoclave cycle 1	3×10^8	<10	8.52
	2×10^8	<10	8.35
	5×10^8	<10	8.74
Suspension for Unisteri SL Autoclave cycle 2	8×10^8	<10	8.95
	7×10^8	<10	8.89
	6×10^8	<10	8.82
Suspension for Unisteri SL Autoclave cycle 3	1×10^8	<10	8.05
	4×10^8	<10	8.64
	9×10^8	<10	9

When a certain number of bio-indicators contaminated with 10^6 or higher resistant bacteria spores are inactivated, it can be concluded that when applying the full cycle, an SAL of 10^{-6} is guaranteed at a theoretical spore-inactivation rate of ≥12 lg increments. This corresponds to "Overkill conditions [8-11]. SAL of 10^{-8} indicates a 1 in 1, 000, 00000 likelihood of an organism surviving to the end of the sterilization process.

For Gram positive bacteria, 12 Ampoules *Geobacillus stearothermophilus* was placed in different position of Autoclave Chamber. Three individual solid cycle of each Autoclave was run. After incubated at 60^0C for seven days no growth was found and Log Reduction was calculated (Table 3).

Table-3: Log reduction from initial count For Gram positive bacteria

Suspension No	Autoclave Cycle	Autoclave	Initial population of 12 Ampoules	Population after Sterilization	Log Reduction
1	Solid	Astell SR 100	10^6	0	6
2	Solid	Astell SR 100	10^6	0	6
3	Solid	Astell SR 100	10^6	0	6
4	Solid	Unisteri SL	10^6	0	6
5	Solid	Unisteri SL	10^6	0	6
6	Solid	Unisteri SL	10^6	0	6

A similar attempt were made by Thomas von Woedtke and Axel Kramer [12] with *Bacillus subtilis* spores where mortality curves on the basis of experimental data were maintained in a range of about 8 orders of magnitude. Under this condition, the attainable degree of reduction of the test organisms can be exactly quantified on the one hand, and the actual inactivation kinetics can be depicted on the other, at least in the range that can be recorded using microbiological methods of proof. Additionally, possible inhomogenities of the mortality curves can be taken into account for extrapolation into the SAL area [12].

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Sterility according to a SAL of 10^{-6} should, logically, only still be required for medical devices and preparations that can be subjected to steam or hot air sterilization using the required standard and equivalent procedures. This is because it is possible that a homogeneous linear mortality curve, and thus the sufficiently certain determination of the treatment conditions necessary to guarantee an SAL of 10^{-6}, can be presumed only in thermal procedures.

Other authors who carried out a detailed mathematical analysis of the intrinsic uncertainties of the exponential model of mortality of test organisms reach the same conclusions in principle (while also taking into account thermal inactivation kinetics). They explain that for decades, this evidently inadequate theoretical basis has been adhered to without question, using the argument that, due to extreme safety premiums, the safety of sterilized products in practice is secured by assuming higher contamination rates with extremely resistant test organisms when examining sterilization procedures [13].

CONCLUSION

Sterility assurance level verification is prerequisite for all laboratory and industrial sterilizer. This study was an attempt to calculate the sterility assurance level of two renowned branded industrial autoclave in logarithmic value by both gram positive and gram negative bacteria. For gram negative bacteria, the sterilization cycle successfully kill all 10^8 cfu/ml which indicates its ability to reduce bacterial load up to 8 log reduction. On the other hand, for gram positive bacteria *Geobacillus stearothermophilus* Ampoules containing 10^6 cfu were used and found no growth after sterilization which also suggests its 6 log reduction capability. By logical consideration of all aspects, it seems possible to partially reduce sterility assurance levels without any loss of safety. More study need to be conducted by considering different environment and condition like adjusting SAL by considering different load map of sterilizer or studying SAL for medical device or porous item with different parameter to reach a final conclusion about SAL

REFERENCES

1. Agalloco, J., Akers, J., & Madsen, R. (2004). Aseptic processing: A review of current industry practice. *Pharmaceutical Technology*, *28*, 126-126.
2. Mosley, G. A. (2008). Sterility Assurance Level (SAL): the term and its definition continue to cause confusion in the industry. In *Pharmaceutical Microbiology Forum Newsletter*, *14*(5), 2-15).
3. Nguyen, H., Morgan, D. A., & Forwood, M. R. (2007). Sterilization of allograft bone: is 25 kGy the gold standard for gamma irradiation?. *Cell and tissue banking*, *8*(2), 81-91.
4. Mosley, G. A. (2008). Sterility Assurance Level (SAL): the term and its definition continue to cause confusion in the industry. In *Pharmaceutical Microbiology Forum Newsletter*, *14*(5), 2-15.
5. McFarland, J. (1907). The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association*, *49*(14), 1176-1178.
6. Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *Journal of visualized experiments: JoVE*, *63*.
7. Lingnau, J. (1991). Standard versus non-standard sterilization processes. *Pharmazeutische Industrie*, *53*(8), 771-775.
8. Oxborrow, G. S., & Berube, R. (1991). Sterility testing-validation of sterilization processes, and sporicide testing. *Disinfection, sterilization, and preservation. Philadelphia: Lea & Febiger*, 1047-57.
9. Fitzgerald, K. A., Davies, A., & Russell, A. D. (1989). Uptake of 14C-chlorhexidine diacetate to Escherichia coli and Pseudomonas aeruginosa and its release by azolectin. *FEMS microbiology letters*, *60*(3), 327-332.
10. Haberer, K. (2005). Heisig P. 2.6. 1 Prüfung auf Sterilität, 5.1. 1 Methoden zur Herstellung steriler Zubereitungen. *Kommentar zum Europäischen Arzneibuch, 21st issue. Stuttgart: Wissenschaftliche Verlagsgesellschaft, Govi-Pharmazeutischer Verlag*.
11. Kramer, A., Assadian, O., & Kohnen, W. (2013). GMS Hygiene and Infection Control. *GMS hygiene and infection control*, *8*(1).
12. Bliem, R., & Nowak, W. G. (2004). Inaktivierung von Mikroorganismen durch Sterilisation, Desinfektion oder Pasteurisierung: Neue

mathematische Modelle. *Pharmazeutische Industrie*, *66*(4), 470-477.

13. Bliem, R. F., & Nowak, G. W. (2004). Orị ginale. *Pharm. Ind*, *66*(6), 780-785.