AIT Research

Real-Time Sterilization Microbiology – Role of Flow Cytometry

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ABSTRACT

Sterile medical devices provide critical care and diagnostic applications where sterility is validated by demonstrating the inactivation of reference Biological Indicators (BIs). However, BI's are qualified using conventional culture-based methods that may limit the ability to fully appreciate the bacterial inactivation kinetics of a sterilization process such as vaporized hydrogen peroxide (VH2O2). This timely study reports on potential for use of flow cytometry (FCM) as a real-time enumeration technique that overcomes such limitations. It also provides novel insights into killing kinetics to support and advance sterilization processes.

INTRODUCTION

Sterile medical devices provide critical care and diagnostic applications to patients. Confirmation of sterility is reliant on crude approximate bacterial culture techniques, consequently limiting the opportunity for process improvement (McEvoy & Rowan, 2019).

Flow Cytometry (FCM) is best described as automated microscopy where thousands of cells can be analysed in a second. FCM quantitatively measures the optical characteristics of cells as they pass in single file in front of a focused light beam (Veal *et al.,* 2000). By measuring fluorescence, either natural or induced by use of fluorescent markers, cells may be differentiated based on size, shape or phenotype characteristics.

Flow Cytometry, with informative stains such as Propidium Iodide (PI) and cFDA, can provide significant real-time insights into the microbial inactivation provided by a sterilization process.

METHODS

BIs of *Geobacillus stearothermophilus* were partially (av. 3log reduction) inactivated with VH2O2 sterilization. BI's cultured in TSB for 48h.

Flow Cytometry performed with Syto BC, PI and cFDA. Data Analysis performed using FlowLogic v7.2.1

RESULTS

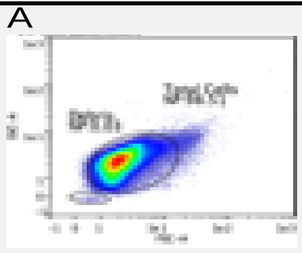
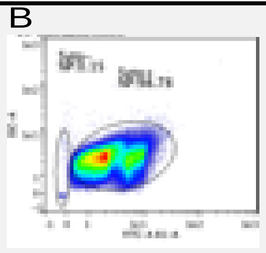
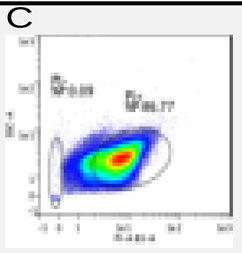
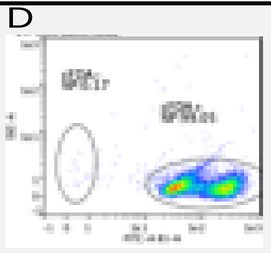
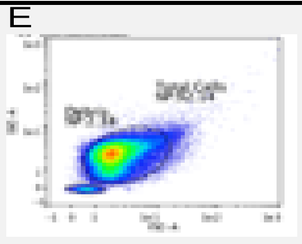
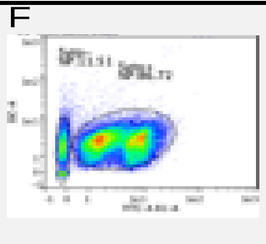
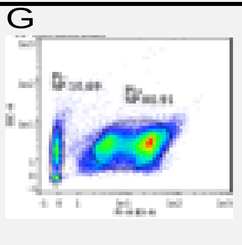
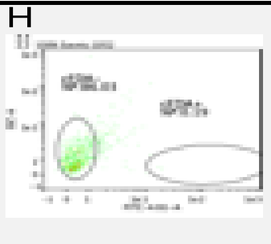
	Forward and Side Scatter	Syto BC	PI	cFDA
	Total Events	Total Cells	Damaged or Dead	Enzymatic Viability
Growth	A 	B 	C 	D 
No-Growth	E 	F 	G 	H 

Figure 1: Flow Cytometry of *Geobacillus stearothermophilus* biological indicators treated with Vaporized Hydrogen Peroxide and incubated in TSB. Plot A-D are of from TSB tubes where growth was observed after 24h. Plots E-H are from TSB tubes where no growth was observed after 48h.

- Population of total cells (Syto BC$^+$) of approximately 8.98 X 10^6 cells of viable (CFDA$^+$) *G. stearothermophilus*, that have damaged inner cell membranes (PI$^+$) (Panel A-D)
- Population of total cells (Syto BC$^+$) of approximately 1.5 X 10^6 cells of *G. stearothermophilus*, that have damaged inner cell membranes (PI$^+$) to such an extent that the cells are not viable (CFDA$^-$) (Panel E-F).
- TSB test-tube of no growth provides valuable FCM data! (Panel E-F)
- Dead cells may be differentiated into two populations (Panel F) of varying levels of membrane damage (Panel G)
- Tube of growing cells show some level of damage (Panel C)

CONCLUSIONS

- Culturing test-tubes of media provide binary information: Growth or no-growth.
- Flow Cytometry on the other hand provides a rich cellular picture of the state of every single cell, dead or alive.
- PI provides information on the damage to the inner membrane - Demonstrated by permeability to PI.
- cFDA can inform if the enzymatic cellular functioning of the cell remains viable.
- Use of these two dyes beneficial in informing on the cellular status of cells not capable of being recovered by traditional cell culture: Dead and Viable but Not Culturable fraction (VBNC) may be analysed.
- FCM can provide valuable information on the transition from viable to nonviable with the inactivation from a sterilization process such as VH2O2.

IMPACT & IMPLICATIONS

- FCM can help in understanding the mechanisms and cellular location of microbial inactivation
- Creating a greater understanding of sterilization process allows for much improved process design, consequently improving medical device performance and ultimately patient outcomes

References

McEvoy, B., and Rowan, N. J. (2019). Terminal sterilization of medical devices using vaporized hydrogen peroxide: a review of current methods and emerging opportunities. *J Appl Microbiol*, 127, 1403-1420.

Veal, D. A., Deere, D., Ferrari, B., Piper, J., and Attfield, P. V. (2000). Fluorescence staining and flow cytometry for monitoring microbial cells. *J. Immunol. Methods*, 243, 191–210.