## To die or not to die, that is the question: the paradigm of sterilization

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Sterilizing, at first glance, seems like a straightforward all-or-nothing process. An item is sterile or it is not. As such, any new technologies developed as alternatives to autoclaving should meet the same requirements: the complete inactivation of a standard load of bacterial spores that guarantees that all microbes will also be killed by the process. A burden of proof is thus imposed on the assessment of sterility, which is based on survival curves. However, the correct interpretation of these curves is of paramount importance, both to elucidate the mechanisms of bacterial inactivation and to be sure that the inactivation is irreversible. How then do we interpret the biphasic nature of some curves, and what are the reasons for and implications of the tailing often observed? When assessing sterilization potential, researchers rely on cell cultures, which are still the gold standard. However, cell cultures rely on the inability of a large population of spores to "revive," that is to (1) germinate, (2) proliferate, or (3) produce "visible" colonies on nutrient agar. Should superdormant spores that require higher priming by germinants be taken into consideration in experimental designs? A good understanding of the mechanisms involved in the inactivation of spores and vegetative bacteria by plasma-based technologies is thus vital, from the design stage through to the approval of new types of sterilizers. Is irreversible blocking of spore germination an indication of successful sterilization? How can this be assessed? And, how specific should the sterilization target be? Logically, the more specific the target, the fewer the number of susceptible microorganisms. Targeting DNA is a good example. Spores and vegetative bacteria can both repair damage to their DNA very quickly. However, since their DNA is damaged in different ways, they use different strategies to repair the damage. In addition, some microorganisms are more efficient in repair than others. This thus raises another question: are spores the best biological indicators when DNA is targeted? My goal is not to provide precise answers to the questions I have raised, but to lay a foundation for discussions and reflections on key sterilization paradigms that have an impact on experimental designs and, ultimately, on the acceptability of new technologies as alternatives to current standard sterilization processes.