Results interpretation after a chemical decontamination cycle performed with vaporized hydrogen peroxide

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This article discusses the challenge of the unexpected growth of a positive biological indicator in a chemical decontamination cycle. Specific features to be considered regarding biological indicators used for the validation of decontamination cycles are presented. Some practical solutions are suggested to avoid the risks related to false positives.

Biological indicators

An undesiderable event that might have happened to many, is the unexpected growth of a biological indicator (BI) at the end of a decontamination cycle when, a positive result is highly improbable. How best to face this situation? If a single BI were used to provide answers for each position, a deep analysis of the failing cause would not be possible, thus maintaining the dilemma: “is it an inadequate cycle or a defective BI?” In that situation, even a statistical analysis would be unfeasible. But, the application of, at least, two or three BIs per point might help overcoming the difficulty.

Hydrogen peroxide

Biological indicators produced and used to validate a chemical decontamination cycle, as with hydrogen peroxide, have specific features. Being a surface agent, the vaporized hydrogen peroxide is not able to penetrate the deeper layers of the material unless the indicators form a mono-layer of spores deposited on the chosen substrate. This way, the biological indicator spores on the substrate are easily reachable by the chemical agent making possible to assess its microbiological effectiveness. Otherwise, the peroxide would be effective only on the top layer of spores with the risk of false positives at the end of the incubation period.

BIs preparation

The inaccurate preparation of BIs or inappropriate conservation storage conditions could entail the deposit of accumulated spores unreachable by the hydrogen peroxide. Therefore, the application of more than one BI per position becomes beneficial to help determine the cause of a positive output and to evaluate the reduction of the microbial level reached. For example, considering a position where 3 (three) BIs had been placed and supposing that the result obtained was: + - - , the challenge in this case lies on the attribution of the positive result to the BI rather than to the cycle conditions.

At this point, it becomes fundamental to repeat the cycle to confirm the result. For this specific case, the Halvorson-Ziegler equation may be applied. It allows the calculation of the Most Probable Number (MPN) of microorganisms surviving in a positive sample with confirmation of the Spore Log Reduction (SLR). The equation can be statistically applicable only with the use of multiple BIs per location.

How to apply it?

The Halvorson-Ziegler equation is the following:

MPN = ln (n/r), where:

ln = natural logarithm function
n = number of replies per point
r = number of negative results per point

Applied to the above-mentioned case (+ - -):

$$\mathbf{MPN = \ln (3/2) = 0{,}405}$$

It is possible to correlate the Most Probable Number (MPN) to the initial population of inoculated spores in order to determine the Spore Log Reduction (SLR) achieved during the cycle. The obtained SLR is actually a result of the following equation:

$$\mathbf{SLR = Log_{10}\, N_0 - Log_{10}\, MPN}$$

where:

N_0 = initial quantity of spores for each inoculated substrate

From which, if N_0 were equal to 2.8×10^6

SLR = 6.447 - (- 0.393) = 6.840 SLR achieved in the case (+ - -).

It is important to highlight that if the obtained result were, for example, (+ + -), the SRL achieved would correspond to 6.406.

In both cases, despite one or more positive growth results, the SRL calculation is able to meet the requirements, for example of the U.S. Pharmacopeia (USP), which defines a decontamination level of 3 to 4 logarithm reduction in spore population as acceptable performance. On the contrary, if a single BI were placed and a positive result achieved, it would not be possible to determine the logarithm reduction and whether to accept or not the microbial result.

Final considerations

The practice of several BIs positioned at the same place enables greater clarity as to the cause of positive results and to investigate the hypotheses of false positives. At the same time, it allows the assessment of the reduction extent of the microbial load.