Steinemann DISINFECTION

Technical Note

Classification of biological indicators with *Geobacillus stearothermophilus* for verifying aerosol-based hydrogen peroxide disinfection systems in the context of EN 17272

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1 Test concepts: Biological indicators versus EN 17272

The verification of automated aerosol-based disinfection processes can essentially follow two different testing philosophies. On the one hand, verification may be performed using biological indicators, typically with *Geobacillus stearothermophilus*; on the other hand, it may be performed using standardised efficacy testing in accordance with EN 17272:2020 [1].

Biological indicators consist of highly resistant bacterial spores and are deliberately used as a standardised “worst-case” challenge. In simplified terms, they are used to assess whether a process is sufficiently powerful to reliably inactivate even particularly resistant microorganisms. If this hurdle is overcome, it can be inferred that less resistant microorganisms will generally also respond sensitively.

EN 17272, by contrast, follows an organism-specific approach. It tests efficacy against defined reference organisms, including *Staphylococcus aureus*, *Enterococcus hirae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *Aspergillus brasiliensis*, *Mycobacterium terrae*, *Mycobacterium avium* and viral models such as murine norovirus and adenovirus type 5 [1].

The standard requires clearly defined reduction performances, typically ≥5 $\log_{10}$ for bactericidal efficacy and ≥4 $\log_{10}$ for other microorganism classes [1].

The fundamental difference is therefore that biological indicators primarily address **maximum process performance**, whereas EN 17272 demonstrates targeted efficacy against specific microorganisms.

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2 Significance and quantification of biological indicators

Bacterial endospores such as *Geobacillus stearothermophilus* are among the most resistant microbial structures. This resistance is based, among other factors, on structural properties such as low water content, stable protein structures and protective chemical components [4]. These spores are therefore particularly suitable as a conservative test marker.

One often underestimated advantage of biological indicators lies in their **quantitative significance**. Commercial biological indicators are standardised and contain a defined spore population, typically in the order of 10^4 to 10^6 colony-forming units (CFU) per carrier [18][19].
This defined initial load allows direct linkage to $\log_{10}$ reduction.

In simplified terms, this means:
A biological indicator with an initial load of 10^6 spores represents a **6-$\log_{10}$ challenge**. If it is fully inactivated, with no growth detectable after incubation, this corresponds to a reduction of at least 10^6, i.e. ≥6 $\log_{10}$. By analogy, an indicator with 10^4 spores can be interpreted as a 4-$\log_{10}$ indicator.

In practice, this significance is further supported by statistical effects. Biological indicators are produced in batches, with each batch having a defined mean population, for example 1.7×10^6 spores per carrier [19]. This specification describes a mean value with natural variability.

If several indicators of the same type are used in parallel, this creates robust empirical confirmation. For example, if ten biological indicators with a nominal load of 10^6 spores are used and all ten show no growth after incubation, it is highly justifiable to assume that the reduction actually achieved fully covers not only the nominal value but also the real mean spore load (e.g. 1.7×10^6).

Each individual indicator already represents a demanding test. Several indicators successfully passed at the same time act like a “repetition of the same experiment” and significantly increase the confidence of the statement.

It is important, however, that this interpretation is based on an indicative, statistically supported assessment and is not exactly identical to the formally defined $\log_{10}$ determination in standardised suspension or carrier tests. Nevertheless, it provides a practical and conservative estimate of the disinfection performance achieved.

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3 Classification in relation to EN test organisms and practical interpretation

The use of biological indicators allows a robust assessment of **maximum process performance**, particularly with regard to sporicidal efficacy. From this, conclusions can be drawn regarding other microorganisms whose resistance is generally lower.

Vegetative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* or *Acinetobacter baumannii* are typically considered less resistant than bacterial spores. Accordingly, a successfully passed spore indicator test can be interpreted as strong evidence of bactericidal efficacy [1].

A similar principle applies to yeasts and moulds such as *Candida albicans* and *Aspergillus brasiliensis*. Here too, intrinsic resistance is generally below that of bacterial endospores, although environmental conditions can play an important role [1].

Mycobacteria (*M. terrae*, *M. avium*) represent an intermediate level. They are more robust than many vegetative bacteria, but generally less resistant than spores. Hydrogen peroxide-based processes have demonstrated efficacy against these organisms [8][9][10].

In the field of viruses, a distinction must be made between enveloped and non-enveloped viruses. Enveloped viruses are comparatively sensitive, whereas non-enveloped viruses such as norovirus or adenovirus show higher stability [5]. Hydrogen peroxide also shows good efficacy against these more robust viruses, provided that the process parameters are suitable [11][12][13].

Despite these clear biological relationships, one central point remains:
The significance of biological indicators is high, but indirect. They demonstrate the ability of a process to overcome a very demanding microbiological challenge. EN 17272, by contrast, requires direct evidence against specific microorganisms under standardised conditions [1].

The combination of both approaches therefore provides the most complete assessment.
Biological indicators enable a conservative, quantitative and practical assessment of process performance, while EN tests provide formal confirmation of specific efficacy claims.

In summary, it can be stated that:
Verification with *Geobacillus stearothermophilus* represents a demanding and conservative test approach which - particularly when multiple indicators with a defined spore population are used - enables a robust estimate of the achieved $\log_{10}$ reduction. In many cases, these results can be transferred to less resistant microorganisms, but they do not replace testing in accordance with EN 17272.

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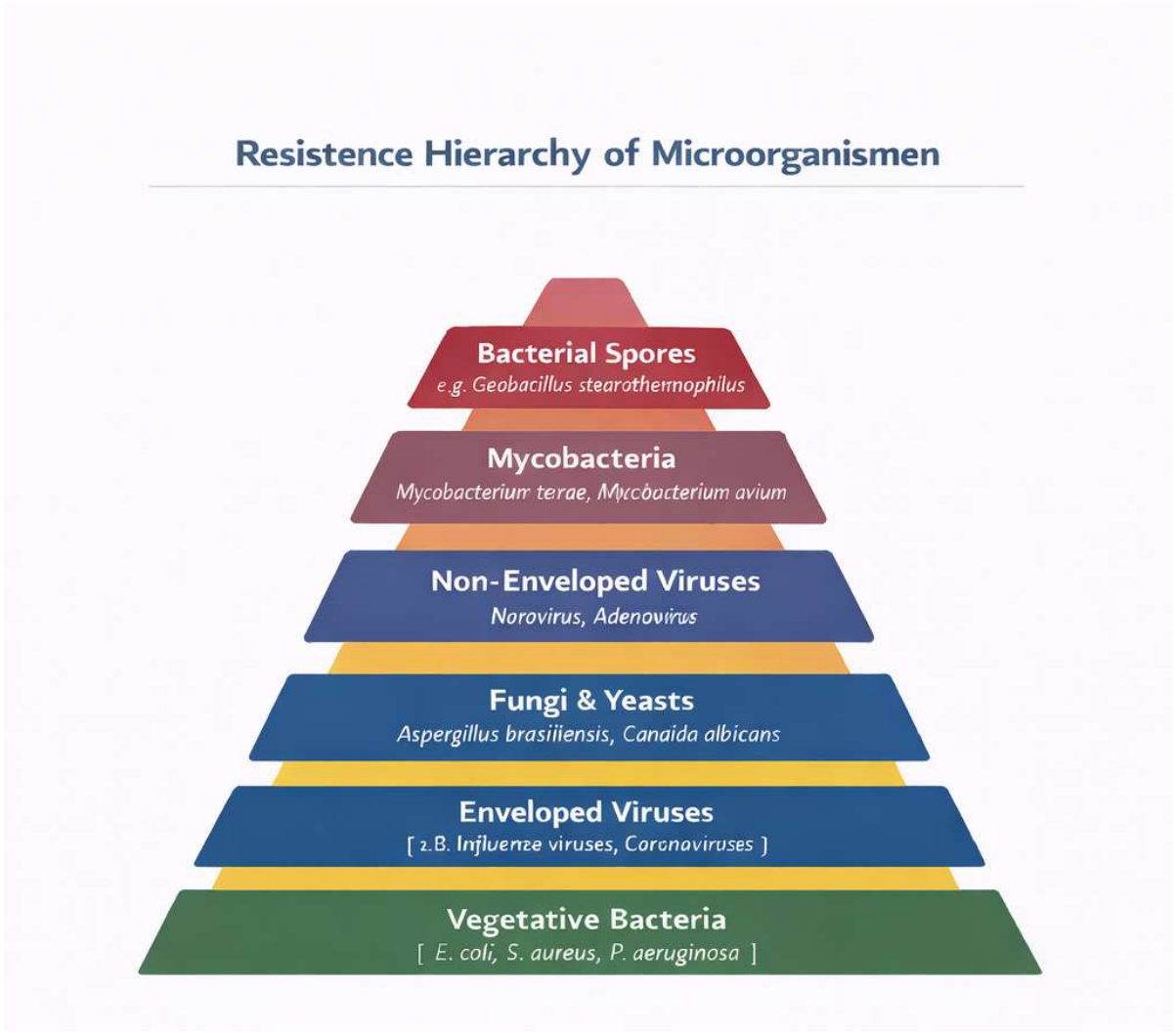

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4 References

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