What's new in standardisation

Current status of standardization for hydrogen peroxide/plasma sterilization processes

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1. Introduction

Hydrogen peroxide vacuum (VH2O2) sterilization processes are used both in healthcare for the sterilization of reusable instruments and industrially for the sterilization of medical devices. There are industrial VH2O2 sterilisers with chamber volumes of more than 1 m^3 that work with higher electric field strengths to inactivate the bioburden, but these are not used in sterilization processes in hospitals.

Often these processes are also referred to as plasma sterilization, although to my knowledge there is no publication that provides evidence that plasma in the form used there contributes to inactivation. On the other hand, studies have shown that the plasma phase has no effect to kill germs [1, 2].

Furthermore, H_2O_2 decontamination processes under normal pressure are routinely used for the disinfection of rooms and ambulance vehicles.

Already in 2000, Schaffer and Pflug [3] tried to determine the resistance of *G. stearothermophilus* in a VH2O2 sterilization process, but had high standard deviations. Tests have been carried out with VH2O2 concentrations of 1.38 – 5.98 mg/l at 50°C and D-values of 37 – 2.38 sec were obtained.

If D-values are determined in the H_2O_2 gas phase, there is in general the problem that H_2O_2 is absorbed on the carrier of the biological indicator and reacts even further after the biological indicator has been removed from the reaction chamber and can therefore falsify the test result.

Until today, there does not exist a DIN, EN or ISO standard for the resistance determination of biological indicators (BI) to monitor VH2O2 sterilization processes. Since 2017 a Chinese standard [4] exists to measure the resistance of biological indicators for VH2O2 processes. The content of this Chinese standard is based on some parts of the biological indicator standard EN ISO 11138-1 [5], the resistometer standard EN ISO 18472 [6] and the standard EN ISO 11140-1 [7] relating to the VH2O2 process. However, the Chinese standard disregards the fact that a test sterilizer fulfilling the requirement of the resistometer standard is not described in practice.

A method for resistance determination in the liquid phase with an H_2O_2/H_2O solution was published by Deinhard et al.[8], which can be reproduced in any laboratory worldwide, where the measurements can be carried out in the liquid phase only, but not in the gas phase. This test procedure fulfils the requirement that the H_2O_2 and the H_2O concentration are kept constant over the entire process time. This publication fulfils all resistometer requirements, however, the inactivation is carried out in the liquid phase which is probably not identical to the gas phase, even if the same temperatures are kept.

The general validation standard EN ISO 14937 [9] is also applicable to the VH2O2 sterilization process. However, its application requires that a reference biological indicator is defined, that its resistance is known and is verifiably higher than the resistance of pathogenic germs.

2. Problem definition

To carry out resistance determinations of biological indicators, the general biological indicator standard ISO 11138-1 [5] describes test procedures carried out in a special test sterilizer, called resistometer, described for each sterilization process in the standard EN ISO 18472 [6]. The standard requires that all critical variables are kept constant so that the D-value can be determined under constant test conditions changing the time only.

However, all VH2O2 sterilization processes used today do not keep the H_2O_2 and H_2O concentration constant over time. The H_2O_2 concentration is continuously reduced, while the H_2O concentration continuously increases. Both variables have an influence on the inactivation of biological indicators.

The resistometer standard EN ISO 18472 [6] contains test procedures and tolerances for VH2O2 resistometers and defines also a stable plateau period like in other sterilization processes where the test is carried out.

Resistometers for VH2O2 processes that keep all critical variables constant, but change the sterilization time only, are under discussion.

Current approaches for the resistance determination of biological indicators are based on commercially available vacuum sterilization processes, which perform a discontinuous injection of hydrogen peroxide solutions. This approach is problematic on the basis of the EN ISO 18472 [6] requirements because the H_2O_2 and H_2O concentration change during the process duration. Due to the discontinuous process, the problem of the fluctuating gas phase concentration is getting worse. The normative requirements for constant process conditions are therefore not met. There are approaches to circumvent the problem by defining a "dose term" using the concentration/time integral. This approach can be chosen as long as the kill kinetics of biological indicators in VH2O2 corresponds to a zero order reaction kinetics in relation to the H_2O_2 and H_2O concentration. However, confirmation of this reaction kinetics according to the current information state is still pending.

Resistance dependence of biological indicators for VH2O2 sterilization processes

In contrast to all other known sterilization processes, where basically the germ used determines the resistance of the biological indicator, the resistance of biological indicators in VH2O2 sterilization and room disinfection processes depends on more influencing variables, making the whole discussion more complex. The following influencing variables are known:

1. Selection of the germ

In the food industry *B. subtilis* or *B. atrophaeus* and in the healthcare sector *G. stearothermophilus* is mainly used providing different D-values.

2. Production method of the biological indicators

By different cultivation and sporulation of the germ, the resistance can be considerably influenced, even if the same bacterial strain is used.

3. Carrier material of the BI

H_2O_2 may react chemically or catalytically with the carrier to form intermediates that better inactivate the germ. Alternatively, H_2O_2 decomposes into oxygen and water without reacting with the biological indicator. The market offers carriers made of glass fibre, stainless steel, PET foil and Tyvek. Similar data are published in the literature [11]. Different carriers strongly change the resistance of the biological indicator.

4. Surface structure of the carrier material used

Roughness of the surface or porosity of the carrier material change the surface, either distributing the germs better or shielding them in porous systems.

5. Purification of the suspension

Organic and inorganic impurities in the suspension originating from spore production should be particularly thoroughly removed. Remaining peptides could otherwise shield the germs, for example, and therefore prevent inactivation.

6. Inoculation on the spore carriers

Germs should be applied to the surface on a single layer (monolayer inoculation) to prevent that overlapping layers are shielding H_2O_2 access to underlying layers.

7. Biological indicator packaging

Germ-tight packaging consists either of non-woven cellulose fibres, PE fibres or combinations of both. H_2O_2 can already react with cellulose fibres and reduce the H_2O_2 concentration inside the packaging. Therefore, cellulose packaging is not recommended in VH2O2 processes. It is also recommended to use naked biological indicators to monitor room disinfection processes. However, there is the problem afterwards of an aseptic transfer into the growth medium solution for the assessment of the biological indicator.

When manufacturing biological indicators for VH2O2 sterilization process monitoring, these points must be considered and make the manufacturing effort more complex than, e.g. manufacturing biological indicators for steam sterilization, where the points 3 to 7 play a secondary role.

3. Current standards and new standardization activities

3.1 Resistance determination of biological indicators

The following standards exist:

- EN ISO 11138-2 for EO
- EN ISO 11138-3 for steam
- EN ISO 11138-4 for dry heat
- EN ISO 11138-5 for LTSF

Currently a standardization project is working to determine the resistance of biological indicators for VH2O2 processes. As described above, a resistometer is missing, the ISO working group is working on an alternative test method (draft prEN ISO 11138-6) [10].

A German working group uses a resistometer under normal pressure with a carrier gas (N2 or air) [2], into which liquid H_2O_2/H_2O mixtures are evaporated. Therefore the necessary constant H_2O_2 and H_2O concentration over time, enabling variable expositions fulfilling the basic requirements according to EN ISO 18472 [6], is achieved. Inside, D-value tests can be carried out with the survival curve procedure according to EN ISO 11138-1 [5]. These measurements produce well reproducible results and can be used e.g. to determine the dependence of the BI resistance on different carriers and the surface coating type (monolayer). It remains to be seen which method(s) for the D-value determination will be included in the standard.

3.2 Standards for the validation of sterilization processes

Today, the general validation standard EN ISO 14937 [9] for all sterilization processes (also called “mother standard”) also applies to the VH2O2 process. However, it does not contain specific instructions for H_2O_2 processes.

Specific standards already exist for the following sterilization processes, which are derived from the mother standard:

- EN ISO 17665 for steam
- EN ISO 11137 for radiation
- EN ISO 20857 for dry heat
- EN ISO 11135 for EO
- EN ISO 25424 for LTSF

in which specific requirements for the individual process are specified.

Currently, an ISO working group is developing a draft standard for VH2O2 processes (draft EN ISO 22441), but requires the standard for resistance determination of biological indicators for VH2O2 processes to be used.

It is necessary that minimum requirements for the inactivation of biological indicators and minimum penetration characteristics are included in the new validation standard for H_2O_2 processes, as is also required in the existing validation standards.

3.3 Standards for sterilizers

European sterilizer standards exist, which are accepted worldwide:

- EN 285 for steam
- EN 1422 for EO
- EN 14180 for LTSF

A European working group is working on a new standard for VH2O2 sterilizers (draft EN 17180) to define the minimum requirements for biological indicator inactivation and their penetration characteristics.

Only when the 3 standard projects for VH2O2 resistance determination of biological indicators, validation of the process and specifications for sterilizer requirements have been completed, VH2O2 sterilization processes, which have already been used in practice for years, will finally be able to work on a generally valid scientific basis and no longer according to the specifications of individual sterilizer manufacturers.

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