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Hydrogen Peroxide (H_2O_2) sterilization validation

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Abstract

The definition of validation is the process of continuously meeting the predetermined conditions that are valid and necessary for all sterilization systems. These conditions include all of the test methods and/or results determined while examining the performance criteria of sterilization devices. Evaluation of the results of the tests performed allows us to prove the effectiveness of the chosen sterilization method by examining the materials expected to be sterilized in terms of bacterial load. The unchanging common point of all sterilization methods is validation studies. The requirements of the ISO 14973 standard should include the evaluation of the sterilization method (sterilization device) performance parameters, as well as the type, content, and effectiveness of the sterilization agent during validation studies. Sterilization The main purpose of validation is to constantly prove that the targeted criteria have been achieved as a result of sterilization. The validation steps are defined as installation evaluation, operational evaluation, and performance evaluation with the most general grouping. In the Hydrogen Peroxide Sterilization method, the operational evaluation should ensure that all the tests defined in the relevant standard of the sterilization device are performed effectively. The installation evaluation should show that the sterilization device is fully assembled by the standard and that the connection to the component sources such as water, air, and steam fed to the device is guaranteed to be faultless. The operational evaluation should ensure that the sterilizer is performed by the operating parameters specified by the manufacturer. Performance evaluation should be made periodically by the users during device use, to prove effective sterilization. As a result, in the validation studies of the Hydrogen Peroxide Sterilization device, the devices to be used in the study should first be operated without load, then biological indicators should be placed in the boiler of the sterilization device by determining the points where the sterilization agent is most difficult to reach. After sterilization, it should be expected to achieve the targeted SAL value. G. stearothermophilus is preferred to provide difficult conditions in hydrogen peroxide sterilization studies.

1. Introduction

With Hydrogen Peroxide Gas Plasma Sterilization devices, hydrogen peroxide, which has a concentration of approximately 60%, is made more concentrated in the boiler of the device under vacuum (85-95%) and is purified from microorganisms. In general, the working steps of the devices are seen in Figure 1 in their most basic form. It is known that when the boiler is ventilated after the sterilization process, the amount of residue is very low (≤1ppm TLV) due to the effect of the plasma unit. The amount of residue must be proven by the manufacturers by performing validation tests [1].

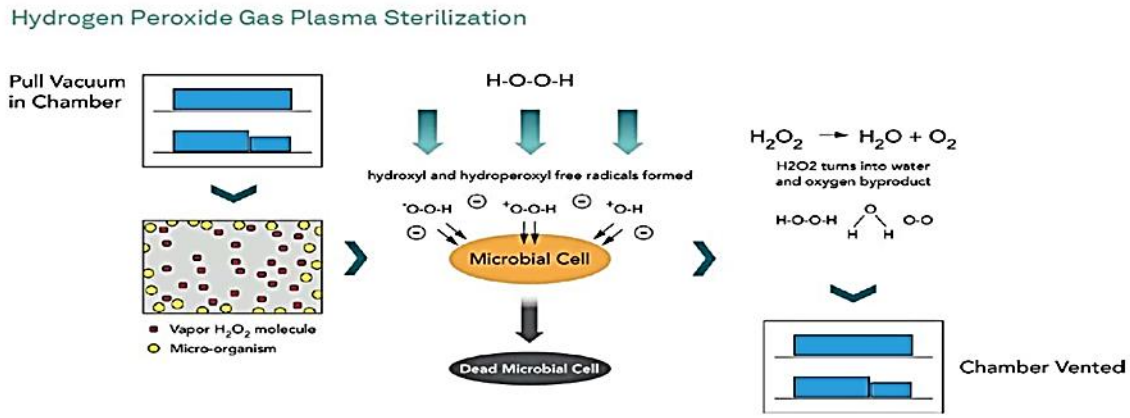

Figure 1. ASP-Advance Sterilization Products [1]

The TS EN ISO 14937 standard defines the requirements for periodic controls of the determined parameters to develop, validate and control the continuity of the selected sterilization method. The working principle of sterilization devices is based on the principle of destroying the microorganisms that may be present on the material and/or materials to the lowest possible level with different applications. In the application area of this standard, some companies produce sterilizers, manufacturers of materials to be sterilized in the sterilization device, and companies that undertake the sterilization process. The standard also defines the basic requirements of the quality management system that will ensure the successful completion of the sterilization cycle. There are two injection times in all cycles of hydrogen peroxide sterilization devices, and there is pressure (torr) variation against time (min) in Figure 2. A standard cycle includes delivery, vaporization pump-down, chamber pump-down, transfer, diffusion, plasma, and vent steps [2]. The parameters (pressure, temperature, time, plasma power, etc.) of all process steps are followed and the devices are expected to work by the set parameters. Validation studies of these parameters are critical for the safety of the entire sterilization process [3].

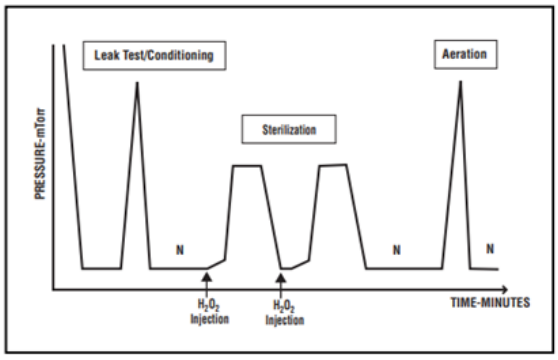

Figure 2. Typical VHP® MD Sterilization Cycle [4]

Hydrogen peroxide H_2O_2, which is used as a sterilization device and a sterilization agent, should be evaluated separately while validation studies regarding method safety are carried out in the Sterilization method with Hydrogen Peroxide. Hydrogen peroxide, which is used as a sterilization agent, has a concentration of approximately 60%, and the storage conditions and shelf life of the sterilization agent are important as they directly affect the effectiveness of sterilization. Therefore, both the concentration amount and the shelf life should be validated. Microbicidal Effectiveness studies of hydrogen peroxide show that Geobacillus stearothermophilus is the most resistant organism to sterilization [3]. Depending on the use of hydrogen peroxide, material effects should be tested and evaluated on the sterilized material/device. The necessary information should be given to the users by determining the material compositions that can be sterilized. The material safety data sheet (MSDS) of the Sterilization Agent should be considered before use. Hydrogen peroxide H_2O_2, which is used as a sterilization device and a sterilization agent, should be evaluated separately while validation studies regarding method safety are carried out in the Sterilization method with Hydrogen Peroxide. Hydrogen peroxide, which is used as a sterilization agent, has a concentration of approximately 60%, and the storage conditions and shelf life of the sterilization agent are important as they directly affect the effectiveness of sterilization. Therefore, both the concentration amount and the shelf life should be validated.

Table 1. Material Composition for H_2O_2 Sterilization Systems [4]

No	Validation Description
1	Hydrogen peroxide specification(concentration)
2	Hydrogen peroxide self-life stability testing
3	Operation parameters
5	Lumen Claims Tests
6	Residual Tests
7	Material Compatibility Tests
8	Software Tests
9	Reference Microorganism Determine (Geobacillus stearothermophilus)

While sterilizing with Hydrogen Peroxide, the types of materials to be sterilized are determined by validation studies and published by the manufacturers. Table 1 contains information about the devices that can be sterilized with hydrogen peroxide sterilizers and the types of materials from which the devices are produced. All tests with the sterilization agent are performed in two steps, before and after sterilization. The absence of toxic effects of hydrogen peroxide was determined by the studies carried out and defined as environmentally friendly.

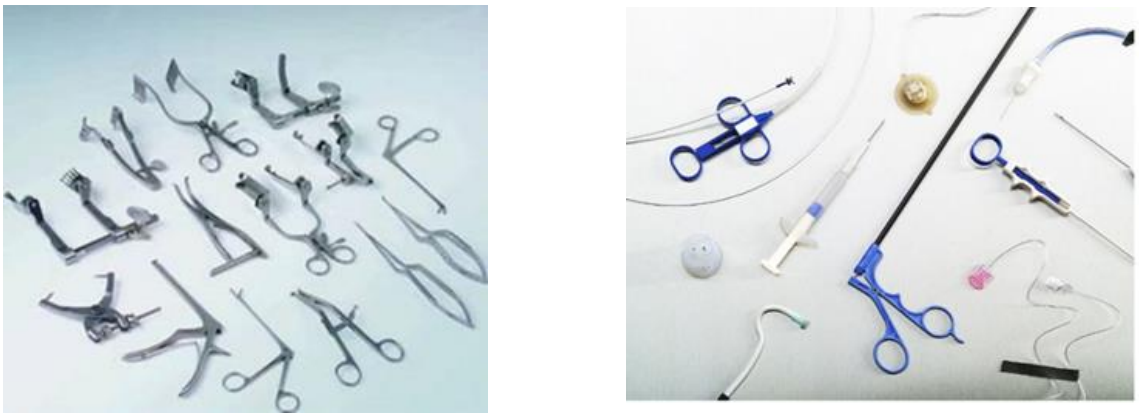

Figure 3. Examples of devices sterilized by Hydrogen Peroxide Sterilization Devices [1]

According to TS EN ISO 14937 standard Article 6 requirements, process and hardware features should be defined and validated. In the validation process of the hydrogen peroxide sterilization device, the following items should be determined primarily;

- Loading plan in which the loading of sterilization is determined.
- Content list of sterilization load
- Thickness, length, diameter, and quantity information of lumen material
- Worst-case scenario (complicated load) definition of lumen material simulation
- Instructions containing the points to be considered while preparing sterilization packs
- The type of biological indicator planned to be used during validation studies
- Layout of the determined biological indicators in the sterilization device in proportion to the sterilization load.
- The quality and quantity features of the kits to be used in validation studies are defined.

Validation studies are basically evaluated in three stages as installation, hardware and performance. In the first stage, the equipment required to perform the sterilization process in a safe and reproducible manner is defined. In the installation, it is important to ensure the efficient operation of the device by providing the resources needed during the assembly phase of the device. The components of the equipment, the materials that make up the components, and the technical drawings are included in the validation file. Performance tests of Hydrogen Peroxide sterilization devices should both prove that the device works without any problems and that the material removed from the device at the end of the sterilization process is completely safe.

When the studies for STERRAD 100NX, one of the Performance Tests of the Hydrogen Peroxide Sterilization Device, are examined, it is seen that single-channel stainless steel materials are used. The study aims to test the effectiveness of the sterilization process such as low diameter and maximum length in difficult conditions and to determine whether the device results in a successful sterilization process. Lumened materials used for this purpose are 0.7mm in diameter and 500mm in length. The middle part of the materials with lumen, which contains a channeled structure on the inside, was determined as the most difficult point for the sterilization agent to reach

and biological indicators were placed in this section. The devices used in performance measurement tests were operated half-cycle, making the process even more difficult [5].

Table 2. Sterrad 100NX Lumen Performance Tests [5]

Load Type	Inside Diameter	Length	Cycle Type
Single Channel Metal Lumen (SS)	≥0.7mm	≤500mm	Standard Cycle
Max 10 lumen per lumen per tray			
PE&TEFLON TUBING	≥1mm	≤1000mm	Standard Cycle
Up to 20 pieces of tubing at one time			
*Flexible endoscopes are excluding Single Channel PE&Teflon Endoscope	≥1mm	≤850mm	Flex Cycle-42 min.

Another important part that needs validation in hydrogen peroxide sterilization devices is software. The sterilization cycles optionally selected by the users for the sterilization devices are automatically managed by the software of the device. The parameters of the sterilization device are controlled by the software.

- Sterilization time
- Working ranges of temperature and pressure parameters
- Injecting the sterilization agent into the device and homogeneously distributing it to the boiler.
- Ensuring safe working parameters of the plasma system
- Giving the necessary signals for the door sensors of the sterilization device to be opened and closed.
- Ensuring the effective operation of the remote monitoring systems of the device

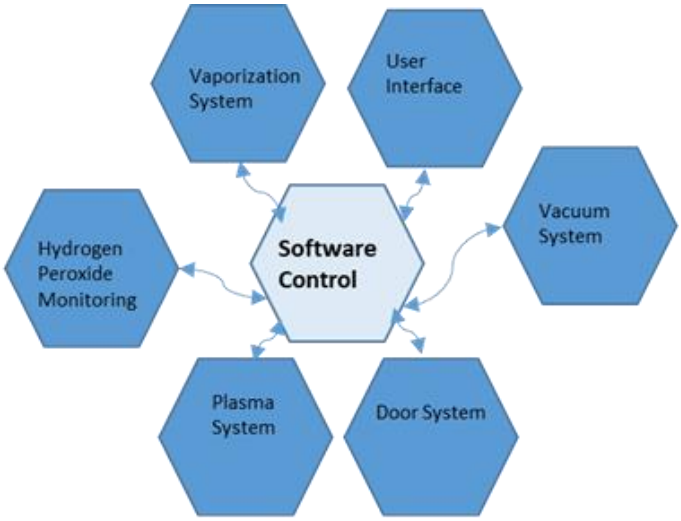

Figure 4. Software Control Components

Each of these parameters can be considered as a process that processes the input signal and converts it into response signals that command the device. The error-free operation of each process is verified by validation studies, allowing the sterilization process to be completed successfully.

2. Results

Effective and successful results are achieved by completing and periodically reviewing validation studies for Hydrogen Peroxide Sterilization devices. All sterilization manufacturers and device users in the world are legally responsible for meeting the requirements for validations and complying with the results. A high number of financial gains are obtained in the sterilization processes performed with the hydrogen peroxide sterilization method. With this method, which does not leave toxic residues, the test results of surgical instruments defined as luminous material (≤1ppm TLV) are successful, as observed in the validation studies of device performance. When

the validation tests performed for Hydrogen Peroxide sterilization devices are evaluated, it has been observed that it is compatible with all material types tested as a result of the sterilization process. When the corrosion tests of the device were evaluated, no corrosive effect was detected for the material types included in Table 2 Sterrad 100NX Lumen Performance Tests [5]. It is defined as a convenient and reliable method for sterilization of such devices. The validation tests to be performed for hydrogen peroxide sterilization devices that provide safe and effective sterilization can be defined as summarized in Table 3. These tests should be added in line with the features of the sterilization devices.

Table 3. Hydrogen Peroxide Validation Tests

No	Validation Description
1	Hydrogen peroxide specification(concentration)
2	Hydrogen peroxide self-life stability testing
3	Operation parameters
5	Lumen Claims Tests
6	Residual Tests
7	Material Compatibility Tests
8	Software Tests
9	Reference Microorganism Determine (Geobacillus stearothermophilus)

3. Discussion and Conclusion

In the packaging validation studies of hydrogen peroxide sterilization devices, a validation protocol is prepared first and the critical process parameters are determined and samples are provided by this study. Optimum conditions are determined by the test results of critical process parameters [6].

By the determined optimum conditions, it is ensured that process controls are carried out before sterilization, after sterilization, and after consecutive double sterilization. A validation report is prepared following the results, and it is reviewed in specified periods. Stability validation work is important for products evaluated within the scope of all medical devices and is carried out by following the items listed below. All of the studies carried out to evaluate all possible risks that may occur in products after sterilization can be evaluated within this scope.

- The scope and purpose of the study are determined by preparing a validation protocol.
- Products are subjected to accelerated aging tests at predetermined periods.
- After aging, sterility and product functionality tests are performed on products.
- Aged package performance tests are applied to packages after aging.
- Sending and recalling products to the furthest target market determined for shipment
- Performing drop tests by the standards defined for the products
- Preparation of the validation report covering the results of the entire validation study

Hydrogen peroxide sterilization devices are preferred more than many low-temperature sterilization methods due to their performance advantages. As a disadvantage, they are not compatible with the sterilization of cellulose-containing components. The Validation Protocol of the studies carried out should meet the following items;

- The purpose of the validation study is to
- The package type of the products,
- Description of equipment,
- Product groups,
- Sampling type/conditions,
- number of samples,
- Parameters (variables)
- Objectives/acceptance criteria

All validation studies performed should be recorded by creating a Validation Report. Validation Reports should include the following titles in scope;

- Summary result,
- Compliance with acceptance criteria,
- The methods used,

• Deviations (if any)

For accelerated aging studies, a special protocol should be created and recorded. The temperature and relative humidity of the cabinets used for the aging of the products should be recorded and monitored. Specific standards for the tests used for the evaluation of sterile barrier systems should be defined and test methods should be clarified. The devices used during physical and microbial tests should be calibrated and recorded with the calibration follow-up list. After aging, package and product performance tests should be repeated and compared with the results before aging. The number of tests required for all validation studies should be considered valid when reliable data are obtained.

As a result, validation studies are the method of verifying with records of a sterilization device or the way the materials sterilized in this device always fulfill the defined requirements with an accurate method.

The aim of the validation studies is to determine and verify the sterilization cycle parameters of the medical devices or materials that need to be sterilized before surgical use. If sterilization validations are done incorrectly, sterilization procedures whose sufficiency has not been proven by tests cannot prevent serious harm to users or patients in contact with the relevant devices in the hospital environment, and fatal errors cannot be avoided.

When hydrogen peroxide sterilization devices are examined, it is observed that there are too many devices produced by different companies, and again, both the designs, material types, and device performances of the devices show differences. Therefore, all different devices and different models of devices should be validated separately. Even minor functional changes in devices require new validation studies.

While performing sterilization validation studies, the verification procedures determined should also comply with the internationally valid ANSI/AAMI/ISO Standards. All studies should be repeatable and provable as a result of different studies.

The standard validation process starts with the placement of sterilization devices or control groups carrying microorganisms in accordance with the determined plan before the sterilization cycles are started.

During the evaluation of all the studies, the level of reaching the defined Sterility Assurance Level (SAL) of the surfaces exposed to the sterilization process is evaluated by the tests performed [7].

In sterilization validation, product vaccination, dosing, sterilization level, bioburden tests, visual control tests, half and full cycle tests, physical strength tests, material compatibility tests, and sterilization level of biological indicators are carried out as general tests.

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Author contributions

Ebru Öner Usta: Conceptualization, Methodology, Writing-Original draft preparation, **Furkan Ayaz:** Data curation, Writing-Reviewing and Editing.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. https://get.asp.com/sterrad-low-temperature-sterilization/
2. McEvoy, B., & Rowan, N. J. (2019). Terminal sterilization of medical devices using vaporized hydrogen peroxide: a review of current methods and emerging opportunities. *Journal of applied microbiology*, *127*(5), 1403-1420. https://doi.org/10.1111/jam.14412
3. Bathina, M. N., Mickelsen, S., Brooks, C., Jaramillo, J., Hepton, T., & Kusumoto, F. M. (1998). Safety and efficacy of hydrogen peroxide plasma sterilization for repeated use of electrophysiology catheters. *Journal of the American College of Cardiology*, *32*(5), 1384-1388. https://doi.org/10.1016/s0735-1097(98)00401-x.

4. Steris Corporation, (2022). Material compatibility with vaporized Hydrogen Peroxide (VHP®) sterilization: A new option for point-of-manufacture sterilization. Case Study 8
5. Diab-Elschahawi, M., Blacky, A., Bachhofner, N., & Koller, W. (2011). Lumen claims of the STERRAD 100NX sterilizer: testing performance limits when processing equipment containing long, narrow lumens. *American journal of infection control*, *39*(9), 770-774. https://doi.org/10.1016/j.ajic.2011.01.010
6. Jacobs, P., & Kowatsch, R. (1993). Sterrad Sterilization System: a new technology for instrument sterilization. *Endoscopic surgery and allied technologies*, *1*(1), 57-58.
7. Atalay, A., Altakhan, M., & Sağiroğlu, P. (2020). İki Farklı Hidrojen Peroksit Gaz Plazma Sterilizatörünün Lümenli Tıbbi Cihazlardaki Performanslarının Değerlendirilmesi. *Journal of Biotechnology and Strategic Health Research*, *4*(3), 272-276. https://doi.org/10.34084/bshr.798413
8. Usta, E. Ö., & Ayaz, F. (2022). Sterilization packaging with appropriate materials and sterility assurance level. *Advanced Engineering Days (AED)*, *4*, 1-3.

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