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Biological indicators for sterilizer qualification

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Sterilizer qualification is the process of **obtaining and documenting** evidence that a sterilizer, i.e., an autoclave, ethylene oxide chamber, or dry heat oven, is capable of consistently producing sterile products under defined conditions. Sterilizer qualification is a critical part of pharmaceutical, medical device, and laboratory quality systems, ensuring that sterilization processes are effective and compliant with regulatory standards.

Sterilizer qualification steps:

It is recommended to perform a qualification procedure after installing a new sterilizer to ensure that the device is properly set up and safe for use. Steps should be followed as outlined below:

1. Installation qualification (IQ)

The first step of the process involves verifying that the sterilizer and its components are installed correctly. Utilities such as electricity, water, and air supply, as well as other system components and security features, must be confirmed to comply not only with the manufacturer's specifications but with applicable regulatory requirements. It should also be ensured that the appropriate technical documentation accompanies the product and that it is complete and accurate.

2. Operational qualification (OQ)

The aim of this step is to ensure that the installed sterilizer operates as intended within all specified parameters (e.g., temperature, pressure, cycle time). This includes the verification of the sterilizer's measurement and control components, as well as of the uniform distribution of the temperature in the chamber. This is the key stage in which the process is optimized, and its "robustness and reliability" must be assessed under the most challenging conditions.

3. Performance qualification (PQ)

The purpose of this step is to demonstrate that the sterilizer, when used as intended, is capable of consistently producing sterile loads under routine operating conditions. This phase is crucial to determine whether the process can be repeated reliably, and whether the operators are properly trained. Load configuration testing represents a key component at this point. Additionally, the use of biological indicators (BIs) and chemical indicators (CIs) comes into play.

Performance qualification: the use of biological indicators

To ensure that the sterilization process can consistently eliminate highly resistant microorganisms under actual or simulated load conditions, the PQ is carried out using **biological indicators (BIs).**

BIs are standardized test devices that contain a known population of highly resistant bacterial spores, typically *Geobacillus stearothermophilus* for steam processes or *Bacillus atrophaeus* for dry heat and ethylene oxide (EO) sterilization.

BIs should be strategically placed in the most challenging areas of the load to sterilize, i.e. cold spots or tightly packed items, representing worst-case scenarios.

Following the sterilization cycle, the BIs are incubated to monitor for microbial growth. A lack of growth confirms a successful sterilization process, whereas the presence of growth indicates process failure.

No growth	=	**Negative result**	=	**Successful sterilization**
Growth	=	**Positive result**	=	**Sterilization failure**

Reasons to avoid fluorescence readout of BIs in Performance Qualification

Self-contained biological indicators (SCBIs) are designed to provide faster readout times in routine monitoring , for instance through fluorescence-based detection methods. However, the **rapid fluorescent readouts are not recommended for Performance Qualification (PQ) of sterilizers.** This is because they do not meet the regulatory requirements or the level of validation rigor that is expected for such purposes. Below is a detailed explanation:

1. Regulatory background

Regulatory agencies such as FDA, as well as standards organizations like USP, and AAMI require **growth-based methods** for PQ , which rely on conventional BIs with conventional incubation periods to **directly confirm the complete inactivation of spores.** In contrast, fluorescence BIs detect enzymatic activity (e.g., α-glucosidase) or protein markers, which indirectly infer microbial viability rather than evidencing actual spore growth. This indirect detection approach lacks the robustness and historical acceptance that growth-based validation methods offer for PQ.

2. Residual activity and its role in BI fluorescence response

Fluorescence-based BIs incorporate a built-in safety margin by relying on enzymatic activity that may persist slightly beyond the point at which the spores are no longer viable. This design

enhances sensitivity and minimizes the risk of false negatives during rapid readout. However, due to the high sensitivity of fluorescence detection, the system may occasionally register signals from residual enzyme activity or protein fragments remaining after spore inactivation.

These signals do not indicate the presence of viable microorganisms but can be interpreted as positive results in specific contexts. In performance qualification (PQ), where the objective is to confirm the complete inactivation of a 10^6 CFU microbial challenge, such an ambiguous signal, although not representative of a sterilization failure, may introduce uncertainty in the interpretation of results.Furthermore, unlike fluorescence readouts, conventional incubation is capable of detecting slow growing or marginally viable spores arising from borderline cases or marginal cycles, which are critical factors for ensuring accurate PQ.

Understanding the nature of fluorescence responses, particularly in relation to residual activity, is essential for maintaining confidence in the outcome of the qualification process.

3. Limited quantitative data

PQ often requires quantitative data, such D-values, log reductions across various load locations, and assessments process challenges i.e., the impact of a process challenge device (PCD) on microbial inactivation. Fluorescence readouts infer microbial viability indirectly and do not provide direct evidence of actual spore growth. Conventional BIs, in contrast, allow for direct observation of spore germination and growth, thereby enabling the obtention of quantitative data.

4. Fluorescence BIs are not designed for depth, but speed

Fluorescence BIs were developed to accelerate results and enable faster decision-making, thereby streamlining the sterilization monitoring process. Response times have been significantly reduced to 4 hours, 1 hour, 20 minutes, and even as little as 5 minutes or 7 seconds. As such, fluorescence readouts are well-suited for routine load monitoring, assessing cycle compatibility with load materials, and troubleshooting —an approach recommended by some sterilizer manufacturers for quick performance evaluations. However, despite representing a major improvement in sterilization monitoring, PQ is a formal, documented process that demands more than just a rapid result: it requires robust, verifiable data to demonstrate process efficacy.

Comparison of conventional BIs vs fluorescence BIs for Performance Qualification

Aspects	Conventional incubation	Fluorescence readout
Detection method	Directly: growth-based	Indirect: enzymatic activity, protein composition
Regulatory acceptance	Accepted for PQ (USP, ISO, FDA)	Not accepted for PQ —unless validated against a conventional BI under the same load conditions
Result time	24 hours - 7 days	7 seconds – 4 hours
Ideal use	PQ, validation, regulatory compliance	Routine monitoring, fast cycle verification

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