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Microbiological Validation of Radiation Sterilization

Introduction

"Sterile" is an absolute term, but the assurance that any given item is sterile is a probability function. The Sterility Assurance Level (SAL) of a product is defined as the probability of any given unit being non-sterile after exposure to a validated sterilization process. For a product to be CE marked and labelled sterile, it needs to conform to the European Standard EN 556. The SAL defined by EN 556 is 10^{-6} , that is one surviving microorganism per one million products.

The Standard that defines methods of verifying a given SAL for irradiated products is ISO 11137-2-2006 (Sterilization of Healthcare Products – Radiation, Part 2 Establishing the Sterilization Dose).

Dose Setting Methodology

ISO 11137-2-2006 outlines two methodologies for dose setting to achieve a given SAL, described as Method 1 and Method 2. A brief description of the theories and procedures regarding these methods are outlined below. These services are available through Isotron Laboratories.

Method 1

Method 1 is used to determine the radiation sterilization dose necessary to achieve a chosen sterility assurance level (SAL). A standard table of microbiological resistances is used in this approach.

Initial Phase – Bioburden Determination

A minimum of ten samples from three separate batches/lots is required for bioburden analysis. Bioburden analysis should be conducted according to a validated protocol. If no validation protocol is available, Isotron Laboratories will assist in developing one suitable for that specific product. The average bioburden of the product is determined for each batch/lot, the overall average bioburden is then calculated using the 30 samples from the three batches. The bioburden count used for the validation is the overall average unless the average of one of the batches is two or more times greater than the overall average, in which case the highest batch average bioburden is used.

Phase Two – Sub process verification dose experiment

Once the bioburden estimate has been determined the sub process verification dose (to achieve a SAL of 10^{-2}) level can be determined using the reference table in the ISO 11137-2-2006 Standard. A SAL of 10^{-2} is used because the results of sterility tests carried out on 100 samples irradiated at this dose level can, by extrapolation, be used to confirm the dose required for a SAL level of 10^{-6} . The bioburden value in the reference table equal to or greater than the actual average bioburden is used. 100 test samples from a single batch are irradiated at the determined sub process verification dose. This delivered dose can have a maximum variance of $\pm 10\%$. The samples are then transferred to the laboratory in preparation for the sterility testing. Each of the 100 test samples is sterility tested independently. These samples are incubated at 30°C for $\pm 2^{\circ}\text{C}$ for 14 days and the number of positives determined. Periodic checks are made to determine the number of positive tests over the 14-day period.

Statistical verification

Statistical verification of the test is accepted if there are no more than two positive sterility tests from the 100 test samples (ie Validation is satisfactory). The required minimum sterilization dose can then be obtained by referencing the standard distribution of resistance tables in the ISO 11137-2-2006 Standard.



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Any failures require further investigation, an augmented sterilization dose may be required.

Method 2

Method 2 is used to calculate the radiation sterilization dose necessary to achieve a chosen Sterility Assurance Level. Regarding the resistance to radiation of microorganisms as they occur on the product. The process is complex and a number of calculations are used to determine this dose. Only a very brief outline of the procedure is given below. Contact Isotron Laboratories for further details of the process.

Initial phase

A minimum of 280 samples from three separate batches (840 in total) must be available for analysis.

Phase Two

20 product items are irradiated at each dose level from each of three batches. A series of not less than nine dose levels and increasing by 2kGy increments (eg 2, 4, 6, 8,, 18kGy) are used, and each of the delivered doses monitored. All the irradiated product items are then individually sterility tested.

Phase Three

Using the sterility test results from above, the calculation outlined in the Standard is used to determine an initial estimate of the dose required to achieve a SAL of 10⁻². The sterility test results also determine which batch is used for further sterility testing. 100 product items from this batch are irradiated at the calculated verification dose and individually sterility tested.

Phase Four

The results of all sterility tests are used to calculate the required minimum sterilization dose.

Dose Substantiation Methods

ISO 11137-2-2006 also outlines methodology for substantiation of two specific sterilization doses, 25kGy and 15kGy.

This methodology is termed VDmax and operationally is similar to Method 1 as it also requires a bioburden determination, the application of a verification dose and sterility testing.

In performing substantiation the method verifies that bioburden present on product prior to sterilization is less resistant to radiation than a microbial population of maximal resistance consistent with the attainment of a SAL of 10⁻⁶ at the selected sterilization dose.

Initial phase – Bioburden determination

A minimum of ten samples from three separate batches/lots is required for bioburden analysis. Bioburden analysis should be performed according to a validated protocol. If a validation protocol is not available then Isotron Laboratories will assist in developing one suitable for that specific product. The average bioburden of the product for each batch/lot is determined, the overall average bioburden is then calculated using the 30 samples from the three batches. The bioburden count used for the validation is the overall average unless the average of one of the batches is two or more times greater than the overall average, in which case the highest batch average bioburden is used. There are maximum allowable limits of average bioburden.

VDmax	25kGy	1,000 cfu per product
VDmax	15kGy	1.5 cfu per product



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Phase Two – Sub process verification dose experiment

Once the bioburden has been determined the sub process verification dose (VDmax dose) is determined from the table in ISO 11137-2-2006 and applied to 10 samples of one batch. The verification dose is conducted at an SAL of 10⁻¹.

The ten samples are then sterility tested independently.

Statistical verification

Statistical verification of the test is accepted if there are no more than one positive sterility test is obtained from the 10 test samples. Then the selected sterilization dose is substantiated. If two sterility test failures are obtained then a confirmatory verification dose experiment must be completed.

Confirmatory verification dose experiment

A further 10 product items from a single batch of product must be selected. The ten items are irradiated at the original verification dose (VDmax dose).

The ten samples are sterility tested independently. If there are no positive tests then the selected dose is substantiated.

Routine Sterilization Dose Audits

Routine sterilization dose audits are required to demonstrate the continued effectiveness of the established sterilization dose. Sterilization dose audits are conducted to monitor the radiation resistance of the bioburden on product.

One of two approaches can be taken to determine the interval of time between dose audits.

- Select an interval of three months.
- Prepare and document a rationale for the selection of time interval between the performance of sterilization dose audits.

There are certain criteria to be met concerning the results of previous sterilization dose audits and the stability of the product bioburden. The maximum allowable time interval between sterilization dose audits is twelve months. Contact Isotron Laboratories for further guidance.