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Radiation Sterilization of Aseptically Manufactured products

Abstract

This technical report addresses a method for validating a sterility assurance level (SAL) of 10^{-6} for aseptically processed products after these products are in their final packaged state. The process involves gamma irradiation of the aseptically processed product at very low doses of radiation, which is possible due to the extremely low levels of bioburden that may be present on the product following a fill-finish operation. Rather than sacrificing a large number of product units to validate the process, the test unit is a surrogate consisting of actual pharmaceutical product that was inoculated with a highly radiation resistant challenge microorganism. Selection of the challenge microorganism was based on analysis of environmental monitoring data taken from an aseptic area. The poster addresses selection and preparation of the surrogate, sterility testing of the surrogate following irradiation in a gamma irradiator, determination of the radiation resistance of the challenge microorganism and application of a method that validates an SAL of 10^{-6} at doses much less than 10 kGy. At these low doses, many types of pharmaceutical products are expected to respond favorably.

Selection/Preparation Test Product

- A library of information on microbial contaminants obtained from environmental monitoring of an aseptic area was evaluated and based on this information a challenge microorganism was selected for testing.
- An inoculum of the challenge microorganism was obtained and prepared for the tests.
- Actual pharmaceutical product was inoculated with the challenge microorganism. The surrogate consisted of 3 ml pre-filled normal saline flush syringes containing sterile preservative free 0.9% Sodium Chloride, USP water. These syringes were inoculated with the challenge microorganism.

Challenge Microorganism Test Conditions

- A spore suspension of *Bacillus licheniformis* (ATCC 14580) was used to inoculate the pharmaceutical product samples. This microorganism, which was found as a common contaminant in the aseptic area, is a Gram-positive spore

forming bacterium that belongs to the *Bacillus subtilis* group of the *Bacillus* genus.

- A total of twenty samples were inoculated for each of the test irradiations. The syringes were maintained in a refrigerated state prior to shipment to the irradiator, during shipment to and from the irradiator and while in storage at the irradiator.
- An additional five inoculated samples accompanied the first set of samples that were sent to the irradiator. These five control samples, which were not irradiated, were counted upon return to the laboratory.

Determination D_{10} Value

- The Stumbo, Murphy, Cochran equation was used to calculate the D_{10} value. Based on fraction negative data,

$$D_{10} = \frac{D_i}{\text{Log } N_o - \text{Log } N_f} \quad (1)$$

D_i = dose delivered to samples

N_o = initial number of microorganisms in colony forming units (CFU)

$N_f = \ln (n/r)$

n = total number of samples irradiated at a dose D_i

r = number of samples that show no growth (fraction negative)

- The ability of the Stumbo equation to predict a unique D_{10} value was tested by conducting multiple irradiations with different values for N_o and D_i .

Results

Test	N_0 CFU	D_i kGy	r	D_{10} kGy
1	1.4×10^4	7.8 ± 0.3	7	1.9
2	1.2×10^4	9.6 ± 0.4	12	2.2
3	3.5×10^4	8.2 ± 0.4	15	1.6
4	3.5×10^4	10.1 ± 0.3	18	1.8
Average D_{10} Value				1.9

Application of Method

The dose that is required to achieve an SAL of 10^{-6} will depend on the radiation resistance of the challenge microorganism and log reduction in bioburden that is required to go from an initial value (N_i) to a value of 10^{-6} . This dose can be expressed in the following manner,

$$D = D_{10} (\log N_i - \log 10^{-6}). \quad (2)$$

A conservative estimate for N_i can be based on results of a media fill consisting of 1000 units. If there are zero contaminated units following sterility testing, it can be conservatively assumed that the initial bioburden N_i is 10^{-3} . This level of contamination is consistent with the definition of an aseptic area wherein the contamination rate is less than 0.1% at a 95% confidence level. At an initial bioburden of 10^{-3} and a D_{10} value of 1.9 kGy, the dose to validate an SAL of 10^{-6} is,

$$D = 1.9 \text{ kGy} (\log 10^{-3} - \log 10^{-6}) = 5.7 \text{ kGy}.$$

Many types of pharmaceutical products may be radiation compatible at this low dose.

