



## **Sterile Cleanroom Management**

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When manufacturing in an aseptic environment, it is critical to ensure that the various cleanroom consumables, such as wipers, gloves, swabs, tubing, etc., will not compromise your environment or products with their presence or use. This is particularly important if the final product's sterility is dependent upon aseptic processing, rather than sterilizing the finished product. In an aseptic environment, entry of a contaminated consumable product could cause your manufacturing process to fail. It is important to understand the sterilization methods used by your consumables suppliers and to ensure that their processes have been validated.

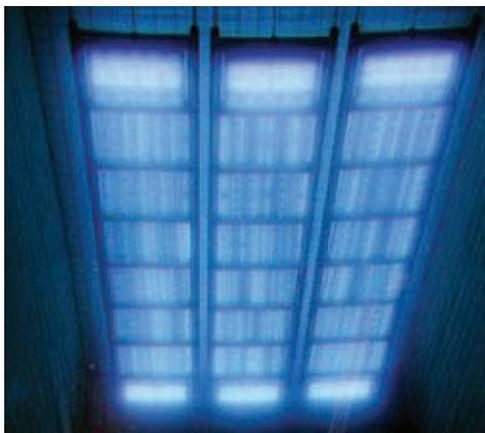
There are various methods available to render a product sterile, with autoclaving, e-beam, and gamma radiation being commonly used in the industry. Each sterilization method has advantages and disadvantages that make it suitable for certain applications and unsuitable for others.

Steam autoclave uses steam and pressure to sterilize a product. A disadvantage of this approach is that many products will have to be repackaged prior to autoclaving, as the packaging used during sterilization must be permeable, creating additional time and expense for the process and product. However, autoclaves are available at reasonable prices so it can be an appropriate option for sterilization within one's own facility, especially when sterilizing small batches.

Electron beam sterilization (e-beam) has a relatively low penetrating power, limiting its utilization to low-density products.

Gamma radiation has a higher level of penetration and is a preferred method for high-density products and/or large batches. It effectively kills microorganisms throughout the product and its packaging, with little temperature variation. Permeable packaging is not required due to the penetrating nature of gamma rays. It is widely used in a variety of industries including food, cosmetics, medical devices, and pharmaceutical products.

Gamma rays are generated from Cobalt-60 sources and are particularly good at ionization. Ionization is radiation with sufficient energy to remove orbital electrons from atoms or molecules, yet not sufficient to induce radioactivity in the product. Ionizing radiation has a lethal effect on microbial life. Because there is minimal variation from batch to batch, gamma radiation is a reliable sterility process (see Fig. 1).



**Figure 1. Gamma rays are generated from Cobalt-60 sources. Photo courtesy of STERIS Isomedix Services.**

## IRRADIATION ALONE IS NOT ENOUGH

The words “irradiated” and “sterilized” cannot be used interchangeably. Irradiation alone is not recognized by the Food & Drug Administration, or by many pharmaceutical companies, as proof of sterilization. Irradiation simply means that the product was exposed to gamma rays. It does not provide any validation that the product received a sufficient dose to achieve sterility, and a Sterility Assurance Level (SAL) cannot be calculated.

ANSI/AAMI/ISO does not recommend using biological indicators for validation and process monitoring. Likewise, sterility testing cannot be used to substantiate a SAL of less than  $10^{-2}$  (i.e.,  $10^{-3}$ ,  $10^{-4}$ , etc.) because of the high numbers of test samples that would be tested. A SAL of  $10^{-6}$  is the probability of finding one non-sterile unit in one million. In order to prove a SAL of  $10^{-6}$ , one million items would need to be sterility tested after exposure to the sterilization process. Obviously, this is not practical.

ANSI/AAMI/ISO has established global standards that can be used to validate a sterilization process, providing the necessary documentation to substantiate your methodologies and processes. Various methods exist to best fit the product and its properties. One that is widely used is 11137. The European Union (EU) has also established standards (EN552), which must be taken into consideration when selling globally. Fortunately, there is a good agreement between the ANSI/AAMI/ISO standards and the EU standards. The validation process must consider the product’s raw materials and components, the microbial barrier properties of the packaging, and environmental controls in place for the manufacturing, assembling, and packaging of those products.

Before starting the sterility validation process, it is necessary to qualify the product and packaging’s ability to withstand the irradiation method. This is necessary because some materials lose strength or discolor when exposed to irradiation, some to the point where they would be unacceptable for their intended use. This can be done by exposing the product and the packaging to various levels of irradiation and testing them against the specifications to determine its maximum tolerated dose. If packaging is purchased from an external source, it is recommended that you obtain the information regarding seal integrity and packaging compatibility from the supplier. Most packaging suppliers will be able to provide a Certificate of Conformance or Analysis.

## STANDARDS AND GUIDELINES

There are several ANSI/AAMI/ISO guidelines which may be followed to validate a sterilization process. The standard that is chosen is one that best fits your product and production processes to obtain the desired SAL. ANSI/AAMI/ISO 11137 Method 1 is commonly used for routine production and involves establishing a sterilizing dose using a bioburden resistance model. There are also options for infrequent production and substantiation of a 25 kGy minimum dose. The SAL must also be chosen and is typically dictated by the product’s intended use. The commonly accepted SAL for an invasive medical device is  $10^{-6}$  which is the highest SAL in use.

## PERFORMING THE INITIAL VALIDATION

For this article we will concentrate on ANSI/AAMI/ISO 11137 Method 1. A bioburden study is performed on ten samples from each of three lots of product, which are randomly selected immediately prior to sterilization. If the item is very large or very costly, a sample item portion (SIP) may be tested for bioburden and the result corrected for the entire product. Bioburden tests involve the removal, culture, and enumeration of viable organisms. One hundred samples of the product are then irradiated at the verification dose that will give a SAL of  $10^{-2}$ . Sterility testing is performed on the 100 samples, and if there are no more than two positives (nonsterile) the validation is considered acceptable. A routine SAL sterilization dose can be calculated based upon the original bioburden results. ANSI/AAMI/ISO 11137 provides a table that lists the required dose to achieve a selected SAL based on the bioburden of the product.

## QUARTERLY AUDITS

ANSI/AAMI/ISO Standard 11137- 1994 recommends that you perform audits at three-month intervals to reaffirm the sterilization dose. The prescribed procedure is to randomly sample 110 product units from a lot immediately prior to sterilization. Ten product units are tested for bioburden and 100 units are subjected to the validation dose ( $10^{-2}$ ) that was determined in the original dose setting. Auditing verifies the process by checking for bioburden changes that may be caused by seasonal fluctuation, raw material and components, changes in personnel, or the environment. Changes in the organisms' resistance to radiation may also affect the validation. Quarterly audits should also include some type of packaging integrity testing (e.g., Burst Test, Methylene Blue Dye Test, or Microbial Challenge).

## BIOBURDEN RECOVERY AND BACTERIOSTASIS/ FUNGISTASIS TESTING

Several other tests are required as part of the initial validation. The bioburden procedure must be evaluated for ability to recover organisms. One method to determine the efficiency of the bioburden sampling method is to inoculate sterile units with a known population of bioburden. The sampling method is performed and the recovery percentage is determined. The recovery factor is then used to adjust the bioburden counts. For example, if the bioburden recovery factor is 80 percent and the initial bioburden count is 150, then the count is adjusted to 188 ( $150/0.8$ ). Another approach is to perform a repetitive recovery method where the extraction method is repeated until there is no significant increase in the recovery of microorganisms.

The USP Bacteriostasis/Fungistasis (B/F) test verifies that the product does not inhibit microorganism growth, eliminating the possibility of a false negative in the sterility test. The standard sterility test is performed by adding a low level of selected microorganisms onto the product. The results should show positive growth within seven days. Growth in the test samples shows that there are no inhibitory substances in or on the test product.

## IRRADIATION FACILITIES

There are many factors to consider when choosing an irradiation facility to process your product. The type of product you have, the cost of the irradiation, and the proximity of the irradiator to your own facility are all factors in making your decision. You should choose an irradiation facility that is cGMP and ISO9000 compliant. A supplier audit is also recommended so that you are assured they will be able to meet all your requirements. The irradiation facility will need to perform a dose mapping on your product to determine the locations on a skid or container, where the minimum amount of radiation is absorbed and where the maximum amount of radiation is absorbed. Dosimeters are placed in many locations, typically at each axis of the packaging configuration. Based on the information from the dose mapping, a loading pattern will be developed for your product (see Fig. 2). The loading configuration is detailed in a customer specification and this pattern is maintained for each load. Two dosimeters, placed in the predetermined minimum and maximum dose locations, will be used for every load.



**Figure 2. Totes entering an irradiation chamber according to a predetermined loading pattern. Photo courtesy of STERIS Isomedix Services.**

## LIMITATIONS OF IRRADIATION INDICATORS

It's important to note that the irradiation indicators that are typically used for inventory control and found on some products do not provide a valid indication of sterility. However, they do quickly indicate whether a product has been through a radiation cycle. Typically, the color of the indicator changes from yellow to red after radiation, although it's important to note that a shift in pH may also cause a change in the color of an indicator. For example, exposure to an alkaline may change the red indicator of a package that has been irradiated back to yellow.

A Certificate of Sterility (CoS), which is provided by consumables suppliers, should contain the necessary information to assure that the product has been sterilized through a validated sterilization process. The CoS should state the product and catalog number, the lot number, the irradiation run number, the date of irradiation, a statement of how the product was sterilized and how the sterilization process was validated, the maximum and minimum specified dose, the maximum and minimum delivered dose, and the signature and title of the approver, who is typically a quality representative. The CoS should be maintained as a record of product sterility.

## ENVIRONMENTAL MONITORING

Factors in the environment and process can affect bioburden levels. For this reason, it is recommended that suppliers of sterile cleanroom consumables perform environmental monitoring to track potential contamination sources such as people, air, surfaces, deionized water, equipment surfaces, chemicals, parts, assemblies, and materials. For example, a Rotary Centrifugal Air Sampler (RCS) can be used to sample bioburden in the air. Any microorganisms that are present in the air will be sampled onto a media strip inside the sampler. The media is then incubated in order to numerate the bioburden level.

If you are manufacturing in an aseptic environment, it's important to determine that your cleanroom supplies have been sterilized with a validated process. Simply irradiating the supplies is not enough to ensure their sterility. Require a CoS with each sterile lot to assure that the consumables product has been not only irradiated but that the process has been validated to ANSI/AAMI/ISO standards.