Gamma Sterilisation Validation according to ISO 11137 - Sterilising dose -

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Established in 1989, Medical Group presently has five subsidiary companies specialising in the biomedical field: Medical Coating, Medical Packaging, Medical Biomat, Medical Manufacturing and Medical Lab. All of these organisations are EN ISO 13485 certified.

Medical Lab offers a comprehensive Performance Qualification service for medical devices: coating, cleaning, packaging and sterilisation validations. The purpose of these qualifications is to assure clients that their medical devices comply with European (EC) and US (FDA) regulations.

Medical Lab performs validations and routine tests.

THE VARIOUS TYPES OF STERILISATION

Two industrial sterilisation technologies are mainly used for sterilization of single use medical devices:
- Ionising radiation (BETA and GAMMA rays)
- Ethylene oxide

Sterilisation by ionising radiation (ISO 11137)

Ionising radiation works by energy transfer (the absorption of energy by the target material). Ionisation occurs at room temperature. Treatment depth varies with the type of radiation. Ionising radiation causes breaks in the chromosomal DNA of microorganisms (at the cellular level), which results in their death.

Sensitivity to radiation varies considerably from one microorganism to another. This basically depends on the type of germ (the species or strain). Radiosensitivity is numerically evaluated using the D10 value. This corresponds to the radiation dose required to reduce the initial bacterial population to 1/10th.
- Most bacterial species have D10 values below 10 kGy.
- The more resistant bacteria (spore-forming bacteria) can have D10 values as high as 30 kGy.

The two type of ionising radiations used for sterilisation of medical devices are:
- BETA radiation: an accelerated electron beam scans the products as they pass under the beam.
- GAMMA radiation: γ photons (electromagnetic radiation) are emitted by a radioactive source around which the products pass.

The choice between beta and gamma radiation is based on:
- the density of the boxes or items
- their size / shape
- their quantity
- the compatibility of the material with beta or gamma radiation

Ethylene oxide (ETO) sterilisation (ISO 11135)

Due to its powerful permeating ability, ethylene oxide is able to reach into the major constituents of living matter. The efficacy of the treatment is influenced by several parameters: the relative humidity of the gas and the temperature, the type of device and the gas concentration used. The contact period ranges from one to six hours, depending on the concentration of spores and germs, as well as the composition of the exposed items.

Ethylene oxide sterilisation is effective against all known microorganisms, except prions.
**Compatibility of materials with sterilisation techniques**

**METALS:**
Metals are generally stable under ionising radiation as under ETO.

**CERAMICS:**
Ceramics are usually as stable under ionising radiation, but ionising radiation can induce changes in colour. Influence of ETO on bioactive ceramics or oxide ceramics has to be validated.

**POLYMERS:**
- Ionising radiation: ionising radiation degrades the properties of most polymers. The type of polymer, the dose received and the dose rate are the main factors influencing the type of degradation observed.
- Ethylene oxide: Minor degradation of PS and its derivative, SAN. Attention should be paid to temperature with regard to polyolefins (PE and PP). The temperature can be adapted. Attention should be paid to humidity with regard to hydrophilic coatings.

**STANDARDS AND REGULATIONS**
The standards applied by MEDICAL LAB for gamma sterilisation validation are as follows:


**IQ, OQ and PQ VALIDATION**

In order to guarantee controlled sterilisation, the sterilisation processes shall be IQ, OQ and PQ validated.

**Installation Qualification (IQ)**
Tests and checks are performed on the sterilisation equipment to verify that their characteristics meet previously established specifications.
Installation qualification (IQ) falls under the responsibility of the sterilisation operator.

**Operational Qualification (OQ)**
After setting the standard adjustment parameters for obtaining a sample result meeting the specifications, the OQ enables validation of these standard parameters, even when the process is carried out under imperfect conditions (adjustment validations and critical cases).
Operational qualification (OQ) falls under the responsibility of the sterilisation operator.

Dose mapping shall be performed based on the distribution and variability of the dose.

**Performance Qualification (PQ)**
This serves to demonstrate that the gamma sterilisation system yields a reproducible, correct result on the product.

**Product PQ**: carried out under the supervision of the product manufacturer. This determines compliance of the sterilization process on the product.

1) **Dose Mapping:**
The first step of the validation is to verify that every product in the sterilization container receives a dose complying with the specifications (for example 25-40 kGy). As the dose received by the products can depend of the density of the products and their position in the sterilization container, before performing the dose mapping validation, the product loaded patter shall be established. With this product loaded pattern, dosimeters will be placed to measure the dose received by the products at different points of the sterilization container.

2) **Validation of the sterilising dose:**
This part of the product PQ makes it possible to validate the minimum irradiation dose required to sterilise the product (i.e. to guarantee a sterility assurance level (SAL) of $10^{-6}$).

3) **Validation of the maximum dose:**
This part of the validation procedure verifies by means of various kinds of tests that product characteristics are not degraded by irradiation, even at the maximum dose.
Establishment of the sterilising dose according to ISO 11137 can be determined by one of the following methods (microbiological methods shall be validated before the analyses are performed). The most widely used method is $V_{D_{\text{max}}}$ method.

**Method 1**: Determining the dose using microbial load information

1. Select the sterility assurance level (SAL) and select 10 product samples from 3 independent production batches (or 30 samples). The samples must be representative of routinely sterilised products.
2. Determine the average microbial load of the 3 batches of 10 items (method based on ISO 11737-1).
3. Obtain the verification dose (referring to table 5 of ISO11137-2).
4. Conduct verification dose experiments on 100 irradiated pieces (method based on ISO 11737-2).
5. Interpret the results.
6. Establish the sterilisation dose based on the results (maximum of 2 positives out of 100 pieces).

130 products are therefore required for this method. The advantage of this method is that it enables any sterilising dose to be validated.

**Method 2A**: Determining the dose using information about the proportion of positives from the incremental dosage in order to determine an extrapolation factor

1. Select the sterility assurance level (SAL) and obtain samples of the product (at least 280 samples for 2 independent production batches). The product samples must be representative of the products routinely sterilised.
2. Conduct the incremental dose experiments; irradiate 20 pieces at incremental doses of 2 kGy beginning with the 2 kGy dose and using at least 9 values. This is to be done for each of the 3 batches involved. Perform a sterility test on each of the products.
3. Conduct verification dose experiments; irradiate 100 pieces at the verification dose and perform a sterility test on each of the products.
4. Examine the results.
5. Establish the sterilising dose based on the results.

This method is seldom used owing to the large number of products and tests required to validate a sterilising dose.

**Method 2B**: Determining the dose using information about the proportion of positives from the incremental dosage in order to determine an extrapolation factor

Applicable if:
- the entire product is tested (SIP = 1),
- after irradiation at any incremental dose, the number of positive sterility tests observed does not exceed 14
- FNP (first non-positive) shall not exceed 5.5 kGy

1. Select the sterility assurance level (SAL) and obtain samples of the product (at least 260 samples for 3 independent production batches).
The product samples must be representative of the products routinely sterilised. 
2- Conduct the incremental dose experiments; irradiate 20 pieces at incremental doses of 1 kGy beginning with the 1 kGy dose and using at least 8 values. This is to be done for each of the 3 batches concerned. Perform a sterility test on each of the products. 
3- Conduct verification dose experiments; irradiate 100 pieces at the verification dose and perform a sterility test on each of the products. 
4- Examine the results. 
5- Establish the sterilising dose based on the results.

This method is seldom used owing to the large number of products and tests required to validate a sterilising dose.

**VD\textsubscript{max} Method:** Justification for a sterilising dose of 25 kGy or 15 kGy

Bellow is an example of the procedure for the VD\textsubscript{max}\textsuperscript{25} method on multiple production batches

1- Obtain product samples. 
The product samples must be representative of routinely sterilised products.
2- Determine the average microbial load of 3 batches of 10 pieces.
3- According to the table of ISO11137-2, determine the verification dose.
4- Conduct verification dose experiments; irradiate 10 products at the verification dose and perform a sterility test on each of the products.
5- Interpret the results: Accept the 25 kGy sterilisation dose if 0 or 1 of the 10 pieces is positive. Conduct verification dose confirmation experiments if 2 are positive. 
Do not accept the verification if there are more than 2 positives.

40 products are required for this method. This method is only viable for validating sterilising doses of 15 or 25 kGy.

**ROUTINE MONITORING AND TESTING: Sterilising dose audit**

Continuous efficacy of an established sterilising dose shall be demonstrated by:
- Periodic Microbial load tests
- Periodic dose audits

In the case of the VD\textsubscript{max}\textsuperscript{25} method, the procedure for dose audits is:
1- Obtain product samples. 
The product samples must be representative of routinely sterilised products.
2- Determine the average microbial load of 1 batch of 10 pieces.
3- Conduct the verification dose experiments; irradiate 10 pieces at the verification dose and perform a sterility test on each of the products.
4- Interpret the results: Accept the 25 kGy sterilisation dose if there is 0 or 1 positive out of 10 pieces. Perform an audit on the verification dose to confirm if there are 2 positives. 
Do not accept the verification if there are more than 2 positives.

The frequency with which sterilising doses are audited must be justified and documented.

According to ISO 11137, the interval between dose audits is 3 months. This frequency can be increased to an interval of 12 months but only with the following justification: there shall be a minimum of 4 consecutive satisfactory dose audits, microbial load determinations shall be made every 3 months, and the medical device manufactured according to ISO 13485 standards.
The various types of tests for gamma sterilisation validation

There are two microbiological tests performed for the validation of the sterilizing dose:
- Microbial load determination (i.e. Bioburden)
- Sterility testing

1. Microbial load determination (Bioburden)


The aim of this test is to determine the number of viable bacteria on the product (before sterilization).

Description of the testing method:

The sample to be tested is immersed in a sterile eluent, which is next divided up and then filtered through membranes placed over selected nutrient mediums and set to incubate at the appropriate temperature.

When incubation is complete, the appearing colonies are counted and the number of micro organisms present on the surface or inside the tested product is calculated. An identification exercise can also be carried out on one or more of the colonies present in order to determine the type of contamination present and thus possibly its origin and its resistance to sterilisation and/or decontamination treatment.
ISO 11737 standard insists on the validation of this technique. Three validation steps are necessary for the validity of routine results to be established.

- First of all, the **elimination of micro organisms** is intended to establish the efficacy of the technique for eliminating germs from the sample in the eluent; the result is a calculation of the correction factor.

- Next, the validation of the **culture conditions** is the basis for selecting the medium(s) used, as well as the temperatures and incubation times which enable the germs obtained from the sample to form colonies.

- Lastly, the investigation of the **inhibitory effect** is to make sure that the type of sample (material, coating, surface residues, etc.) and the physical forces applied during the test do not produce any artefact which could interfere with the microbial load determination.

It is very important to note that an unvalidated test does not comply with standards and has no value.

2. **STERILITY TEST**


The aim of this test is to detect the presence of viable bacteria on the product (after sterilization).

Description of the testing method:
The direct inoculation technique is based upon total immersion of the sample in a culture medium.

Generally, one or two mediums are tested: trypticase soy broth (TSB) to detect the presence of aerobic bacteria, fungi and/or yeast, and TBR (Thioglycollate broth with resazurin) to investigate the presence of anaerobic and aerobic bacteria.

The interpretation is done by turbidimetric analysis: a cloudy medium indicates the proliferation of bacteria. Conversely, a clear medium indicates the absence of growth.

Validation is recommended by the standards in order to guarantee the reliability of the results. It is essential to ensure the sterility and the fertility of the culture medium, as well as the absence of an inhibitory effect on the product to be tested, i.e. the ability of micro organisms to form colonies in the presence of the sample.
DESIGN: QUESTIONS TO ASK

- What are the normative guidelines for the countries where my products are sold?
- What is the list of required tests for my sterilisation qualification dossier?
- Does my sterilisation service supplier have a process performance qualification, an IQ and an OQ?
- What are my product families (refer to the definition of ISO 11137)?
- Which product should I choose to represent the product family during sterilisation validation testing and routine testing?
- Which method should I choose for validation of the sterilising dose?
- Is the sterilisation method compatible with the medical device?
- What are the dimensions of the packaged products?
- What is the density of the packaged products and accepted tolerances?
- Is the packaging material compatible with the sterilization technique?
- Is the product’s initial microbial contamination known?
- Is the type of micro organisms present known?
- If so, how radiosensitive are the germs?
- Can the quantity and type of micro organisms present on my medical devices vary over time?
REASONS FOR CHOOSING MEDICAL LAB

- Acknowledged expertise in medical device testing, and in French, European, Chinese and US standards and regulations
- A full service partner offering training, consultancy and testing services
- An efficient ISO 13485:2003 quality control system
- ISO 17025 certified for Bioburden and sterility tests
- A multilingual sales team (English, German, Italian, Spanish, Arabic, Chinese...)
- Our experts can assist you with your regulatory processes, and help you determine validation protocols
- Our collaborative interaction with MEDICAL GROUP subsidiaries helps you limit your suppliers, save time in having your batches released and reduce your transport costs
- Speedy communication of results (by e-mail or on the website)
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