Go to List

VERIFICATION THE 25-KGY DOSE USING THE ISO STANDARD ISO/TR 13409 FOR THE PRODUCTION OF BONE GRAFTS.

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ABSTRACT:

The sterilization is a validated process used to render a product free from viable microorganisms. For our conditions we preferred the method of substantiation of 25 kGy (ISO Standard ISO/TR 13409**)** because it is designed for small or infrequent product batches (< 1000 product units). It is developed as a means of verifying the use of 25 kGy as the sterilizing dose and is an adaptation of method 1 of ISO 11137. However, ISO TR 13409 establish the SAL for the Verification dose experiment varies depending on the number of product units tested (which is a function of the batch size) and it is very convenient for tissue production.

We performed two principal microbiological activities: bioburden estimate, pre-sterilization count plus a compensation for the efficiency of the recovery method, by ISO 11737-1 and test of sterility by ISO 11737-2. The techniques for Bioburden estimation could be direct or indirect. Choice of direct vs. indirect depends on number of contaminating micro-organisms, product configuration, ability to remove microbial contamination, effect of removal method on microbial viability, types and location of micro-organisms, nature of product and culture conditions. For a test of sterility, he product unit or SIP putted direct immersion in educated culture medium and incubate. Keep precaution to minimize level to the false positives is necessary.

Introduction

Latin America is very underprivileged in the number of radiation sterilized tissue grafts available for clinical applications when compared to developed countries. In USA now a minimum of 640,000 grafts are used annually. The corresponding figure of need in Latin America on a pro rata population basis would be at least 1.2 million. The best production estimate on an annual basis currently is less than 2000 radiation sterilized grafts. However, there are privately imported tissue grafts also. There is clearly a serious shortfall. It can be concluded, that there is a profound need for the IAEA program to stimulate the overall development.

The possibility of infectious disease transmission with tissue allografts is a major concern for tissue banking practice. To minimize the risk of bacterial and especially viral disease transmission, particularly of donor origin, several steps should be taken, including careful donor selection, proper tissue processing and adequate sterilization of tissue allografts.

The use of ionizing radiation for the sterilization of medical disposable products goes back over 20 years being today the sterilization process used by the majority of the manufactures. There are now estimated to be more than 200 industrial irradiation plants around the world, and close of 60% of all medical disposable devices are sterilized by ionizing radiation.

Usually the selection of the sterilization process is based upon the physical and chemical nature of the product to be sterilized. Due the characteristics of biological material, specially the human tissues, the radiation process becomes the more appropriate alternative for the microbial inactivation of these products.

The purpose of this work was to verify the 25 kGy dose using the ISO standard ISO/TR 13409 for the production of bone grafts from the Scientific Orthopedic International Complex Frank País Tissue Bank of Havana City.

Materials and Methods:

1. Bioburden determination

Sample item portion (SIP) selection should be as large a portion of the product unit as is possible to manipulate in the laboratory.

Were selected 10 osseous pieces randomly take account the weight of this. Each one was shacked during 45 minutes at 75 rpm in a bottle containing 50 ml of peptone water 0,1% according to Stracks and Stokes, 1957, and Triton X 0,1% from BDH. The suspensions were filtrated trough membranes of 0,45 μm, was used 20 ml of distillated water to wash each membrane. The membranes were laid on Tryptone Soya Agar.

They were incubated at 32 ± 2^0 C during 72 hours.

2. Extraction efficiency determination

In this case had been used the same method explained before but with successive extractions. The difference is that each tissue unit was suspended twice more in the eluyente for the extraction. For the determination of the extraction efficacy it was used the next equation:

$$\varepsilon = \frac{\alpha}{\alpha + \beta + \chi} \times 100$$

Where α, β and χ are the bioburden after the first, second and third wash respectively

3. Verification of 25 kGy sterilization dose

It was done following the next steps

3.1. Sample item portion (SIP) selection

The size and quantity of pieces needed were selected according to table 8 from ISO/TR 13409. This work was done using a batch with 79 rib pieces.

3.2. Establish the verification dose

Using the average bioburden obtained it was determined the verification dose using the next equation from the standard:

$DV\ (kGy) = I + S.\ (\log X)$

Where:

X: Average bioburden

I: Intercept

S: slope

The intercept and slope values are from table 9 from ISO/TR 13409 of the standard using the average bioburden and the number of pieces needed for the assay.

3.3. Irradiation at the verification dose

There were irradiated 10 osseous pieces at the verification dose calculated. It was used a power self-model PX-χ-30 with Co^{60} sources. The dose rate was of 3.389 kGy/h. The dose mapping of the process and the installation was done using the Fricke dosimeter according to Prieto et al., 1997, while for the routine dose mapping was employed Red Perspex dosimeters (Prieto and Chávez, 1993)

3.4. Sterility test

This test was performed under the right conditions needed for this kind of work to avoid the possibility of false positives. The selected method was the direct immersion of each piece (10) irradiated previously

according to ISO/TC 198 No. 175. The media used were Tryptone Soy Borth and Thioglycollate medium, both suggested in the standard.
The incubation was according to this one and to the Cuban standard NC 26-12:1993 during 72 hours between 32 and 34 degrees and after that during 14 days between 25-27 degrees.

3.5. Irradiation at the sterilize dose 25 kGy
The method used is the pre-establishment of the 25 kGy dose as sterilize dose, then if the sterility was right (it means the number of contaminated sample is not more than the recommended by ISO/TR 13409) it will used this dose to sterilize the batch of the product.

Results and Discussion

The bone grafts are considered medical device having in account that it means any instrument, apparatus, appliance, material or other article, whether used alone or in combination, intended by the manufacturer to be used for human beings wholly or mainly for the purpose of diagnosis, prevention, monitoring, treatment or alleviation of disease, injury or disability, etc and which does not achieve its principal intended action in or on the human body by pharmacological, chemical, inmunological or metabolic means, but which may be assisted in its function by such means.

Bioburden determination

Table 1 show the results obtained from bioburden determination experiment. Tryptone Soy Agar (TSA) was the medium employed according to ISO/TC 198 No.175, because it chemical composition (two kind of peptones and a higher concentration) to recover a high number of microorganisms from the samples is possible.

Table 1: Bioburden determination

Sample	Sample weight	Fcu/sample
1	0.845	3
2	0.953	3
3	0.804	23
4	1.302	10
5	0.849	7
6	0.901	3
7	0.834	2
8	0.820	26
9	0.934	6
10	0.978	20
		103

Extraction efficiency determination

Table 3 shows the results of the bioburden determined for each wash. In the proposed system (medium eluyente) described in Materials and Methods it was obtained and Extraction Efficiency of 82,4 %. With this value the proposed combination is accepted because it is higher than 80 %, as it is recommended by ISO 11137.

It was used this method because the spongy structure with cavities of the bone could have microorganisms and to remove the major quantity of them it is necessary to use an extraction system including a biological detergent and to shake during 45 minutes.

Table 3: Extraction efficiency determination. TSA

Sample	Sample weight	Bioburden by Washes (fcu/U)		
		1	2	3
1	0.845	3	0	0
2	0.953	3	0	0
3	0.804	23	6	0
4	1.302	10	3	0
5	0.849	7	1	0
6	0.901	3	0	0
7	0.834	2	0	0
8	0.820	26	5	0
9	0.934	6	1	0
10	0.978	20	6	0
		103	22	0

The efficiency obtained was:

$$\text{Efficiency} = \frac{103}{103 + 22 + 0} \cdot 100 = 82.4\ \%$$

Bioburden calculation

Is very important know the batch bioburden as exactly as possible. For these objective to determine the efficiency of extraction cells methods is a crucial steps. Any process with efficiency major than 80% is accepted (Tallentire, 1996).

Take account the dates of bioburden estimated and extraction efficiency determinate, to calculate the bioburden more exactly, is possible.

$$\frac{10.3 \text{ fcu/item} \times 100}{82.4} = \mathbf{12.5} \text{ fcu/item}$$

25 kGy sterilization dose verification

The batch had 79 rib pieces the number required for the determination of the bioburden was 10 pieces. It was considered the portion for the assay equal to a complete piece (SIP=1)

Calculation of the verification dose. Irradiation and sterility test.

Sterilization is an example of a process for which efficacy cannot be verified by retrospective inspection and testing of the product. It is important to be aware that exposure to a validated and accurately controlled sterilization process is not the only factor associated with the provision of assurance that the product is sterile and suitable for its intended use. Attention has to be given to the microbiological status of raw material and/or components, the microbiological barrier properties of the packaging and to the control of the environment in which the product is manufactured, assembled, packaged and stored.

A sterile product is one that is free of viable microorganisms. Item produced under controlled manufacturing condition can, prior to sterilization, have microorganisms on them, although ordinarily in low numbers. Such products are, by definition, non-sterile. The purpose of sterilization processing is to destroy the microbiological contaminants on these non-sterile products. The destruction of microorganisms by ionizing radiation follows an exponential law. Accordingly, one can calculate a finite probability of a surviving microorganism regardless of the magnitude of the delivered sterilization dose.

The probability of survival is a function of the number and species of microorganisms present on the product **(bioburden)**, the sterilization process lethality and, in some instances, the environment in which the organisms exist during treatment. It follows that the sterility of individual items in a population of products sterilized cannot be assured in the absolute sense. A sterility assurance level **(SAL)** is derived mathematically and it defines the probability of a viable microorganism on an individual product unit.

Using the average bioburden and the number of samples required for this experiment and with the values of the slope and the intercept from the table 9 of ISO/TR 13409 it was calculated the verification dose and it was obtained the value of 3.1 kGy. With this dose were irradiated 10 bone samples. It was done a sterility test and all samples were sterile.

The verification dose calculated was enough to guarantee a probability of 10^{-1} to found viable microorganisms in the samples. This is valid because it wasn't found any contaminated sample (less than one is allow). It means that the standard applied is suitable for this purpose. Then, the dose of 25 kGy assures the probability of 10^{-6} (SAL) of find viable microorganisms in the batch. This SAL is the required for this kind of product.

Having in account that for each donor is possible to get between 80 to 120 pieces it is possible to use the dose of 25 kGy to sterilize the grafts without risk of contamination for the patient. Furthermore, the use of the irradiation at this dose is supported by many researches about mechanical and biomechanical properties of bones (fragility, pressure, and elasticity) at doses below 30 kGy. In this studies have been proved that this properties aren't damaged with the irradiation. It has been observed affectations at doses of 60 kGy (Mukherjee et. al., 1987). Other studies in relation to osteogenesis and osteoinductive properties of bone grafts have proved there is not significant diminishment (Wientroub and Reddi, 1988)

These investigations have included identification of the origin and stability of radiation induced free radicals and other paramagnetic entities in bone, using EPR spectrometry. It included too the evaluation of the effect of various preservation procedures on the osteoinductive capacity of bone, mechanical properties of bone, resorption rate of tissue allografts in vivo and solubility in vitro and cross-linking of collagen (a major constituent of connective tissue allografts) (Dziedzic-Goclawska A., Stachowicz W., 1997).

Conclusion

We can affirm that 25 kGy dose was verify as sterilize dose for the production of bone grafts in the Tissue Bank ORTOP of the Complejo Científico Ortopédico Internacional Frank País

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Go to List

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