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Gamma Irradiation of Frozen Animal Serum: Dose Mapping for Irradiation Process Validation

By Bart Croonenborghs, Andy Pratt, Lorraine Bone, and Mara Senescu

Abstract

The treatment of animal serum by gamma irradiation is performed to mitigate the risk of introducing undesired microorganisms (viruses, mollicutes, or other microbes) into a cell culture. Serum manufacturers and end-users utilize irradiation contractors to perform this process. The irradiation process must be validated, which involves establishing the: (A) minimum dose that achieves the required inactivation of the microorganisms of interest; (B) maximum acceptable dose at which the serum still maintains all of its required functional specifications; and (C) process used by the contract irradiator that allows treatment of the serum product within these defined limits. In the present article, we describe the best practices for qualifying the distribution and magnitude of absorbed dose (performance qualification [PQ] dose-mapping) when serum is gamma irradiated. PQ dose-mapping includes the following: (1) documentation of dose distribution characteristics in defined product load configurations for a specified pathway through the irradiator; (2) assessment of the process capability of the defined product load configurations and irradiation pathway for respecting the dose specification for the serum; and (3) development of a method for routine dose monitoring of the irradiation process with the defined product load configurations and the specified irradiation pathway.

IMAGE: A cobalt-60 source rack in the storage pool of a gamma irradiation facility. (Courtesy of Sterigenics International, Leuven, Belgium.)

Introduction

This article is one of a series of papers that are being authored under the sponsorship of the International Serum Industry Association (ISIA) with the purpose of establishing best practices for processes employed in the gamma irradiation of animal serum.[1] In the present article, we describe the best practices for qualifying the distribution and magnitude of absorbed dose (performance qualification [PQ] dose-mapping) when frozen animal serum is gamma irradiated.

Routine irradiation of frozen animal serum is typically undertaken at contract irradiation facilities, and not by serum vendors or end-users themselves. The serum is usually irradiated in its final product container, where the containers are placed in specified quantities (with or without cardboard boxes that group several product containers together) at specified positions within a cryotainer, such as a lidded polystyrene box. The cryotainers can be specifically provided for the irradiation process by the operator of the gamma irradiation facility, or they can also be used for transport to and from the irradiation facility.

Dry ice is used to ensure that the cold chain is maintained throughout the irradiation process. The importance of temperature control for preservation of serum performance has been stressed previously in a separate article in this series[2] and will be discussed in more detail in a future paper. Dry ice can be added in specified quantities to specific compartments of the cryotainer, or it can be added in bulk to fill up the entire cryotainer. The former can be done when the cryotainer is specifically designed for that purpose, with the intent to minimize the influence from the presence of dry ice on dose delivered to the serum product. The latter is the most common process, where the cryotainer is also used for transport to and from the irradiation facility. In this case, dry ice is added to the cryotainer in preparation for transport to the irradiation facility, and

an operator at the gamma irradiation facility may replenish the dry ice upon receipt (and/or prior to irradiation), and before the transport from the irradiation facility back to its customer.

Defining, qualifying, and performing an irradiation process for frozen serum presents a number of challenges for a service provider of gamma irradiation. These include the following:

- The above-mentioned requirement to maintain a cold chain during the entire process, from product receipt at the irradiation facility through product shipment from the facility. During the actual irradiation process, the product typically passes through an irradiator for several hours where the temperature can be well above ambient (40–50°C is not unusual).
- The sizable amount of serum product in a product container.
- The large localized density of serum (approximately 1 g/mL) in product containers with empty headspace.
- The customer's requirement for a narrow dose window or specification (*i.e.*, a minimal difference between minimum required and maximum allowed dose for the frozen serum). This can also be expressed as a small dose uniformity ratio (DUR) between the maximum and minimum values.
- The customer's specification of a minimum dose that is greater than the typical sterilization doses used for medical devices. This makes it more difficult to integrate frozen serum into existing processing categories defined for sterilization processes at a contract gamma irradiation facility.

This article provides guidance on how such challenges can be taken into account during PQ dose-mapping for gamma irradiation of frozen animal serum. The material presented is largely based on the standards for radiation sterilization of healthcare products[3,4] and ASTM E2303.[5] The topics to be discussed in the following sections include the: (1) purpose of PQ dose-mapping; (2) prerequisites for PQ dose-mapping; (3) PQ dose-mapping methodology; and (4) analysis and documentation of PQ dose-mapping results.

1. The Purpose of PQ Dose-Mapping

The gamma sterilization of healthcare products is a regulated process.[3,4] The expectations for serum irradiation for the purpose of pathogen reduction are that the facility and the process will have been qualified appropriately, as with regulated healthcare product irradiation. The completion of PQ dose-mapping is one of the requirements outlined in the regulatory documents for sterilization of healthcare products using ionizing radiation.[3,4] The objectives of the PQ dose-mapping study are to:

- Document the gamma radiation dose distribution characteristics in defined product load configurations for a specified irradiation pathway through the irradiator.[3]
- Assess the process capability of the defined product load configurations and the specified irradiation pathway for meeting the specified dose window for the serum product on a routine basis.
- Develop a method for routine dose monitoring of the irradiation process with the defined product load configurations and the specified irradiation pathway.[3]

PQ dose-mapping should be conducted according to a study protocol that should be prepared and approved by the operator of the gamma irradiation facility and the company responsible for the release of the irradiated serum. If an established procedure exists that has been approved by the gamma irradiation facility operator and the company responsible for the release of the irradiated serum, the PQ dose-mapping may be completed using that procedure. The different aspects of the PQ study are described in more detail in the sections to follow. The PQ study is executed per the approved protocol or procedure, and a summary report of the results is prepared and approved by those who have agreed upon them. Based on the information generated in the PQ dose-mapping study, and documented in the summary report, a process specification is prepared for the routine irradiation of the frozen serum product.[3] Guidance on the content of the summary report and process specification is given below.

PQ dose-mapping should be performed in the irradiator where the serum product will be routinely processed, and the irradiation process employed in the PQ study should match, as closely as possible, that which is expected to be used routinely for the irradiation of the serum. Guidance on the irradiation process for PQ dose-mapping is given in section 3 below. The PQ dose-mapping should be repeated if operation qualification (OQ) measurements show that the irradiator has changed to an extent where one or more of the conclusions from the previous PQ are no longer valid, or if there is a change in product that might affect absorbed dose or dose distribution. Such changes might include, for instance, changes in the dimensions of the product containers or the product configuration inside a carton. PQ dose-mapping on a regular basis is not required, but procedures should be put in place for a periodic review of PQ dose-mapping for a given product in order to assess the potential need for repeat of the PQ.

2. Prerequisites for PQ Dose-Mapping

Before the PQ dose-map study method can be defined, a number of prerequisites need to be fulfilled. These include dosimetry system calibration, irradiator qualification, and serum product definition that includes the manner for cold chain management.

Dosimetry System

A dosimetry system must be available, calibrated[6] for the conditions of use, traceable to national or international standards through a non-broken chain of comparisons, and capable of accurate measurement of the doses to be used for the PQ dose-mapping study. Commonly used routine dosimetry systems employ radiochromic film[7], polymethylmethacrylate (PMMA)[8], or alanine[9] dosimeters.

The response of all dosimeters to ionizing radiation is affected by the irradiation temperature.[6–9] For alanine dosimeters, the complex interplay between irradiation temperature, dose, and dosimeter response has been studied by metrology institutes, and results from low temperature studies are shown in **Figure 1**.[10]

The effects of low temperatures on dosimeter response must be considered when performing PQ dose-mapping for product intended to be irradiated at such low temperatures. Either the effects of low temperature on dosimeter response are taken into account during PQ dose-mapping using low temperature product or PQ dose-mapping is undertaken at ambient conditions for which the dosimetry system is more typically calibrated by the provider of the irradiation service.

It is difficult to adequately monitor dosimeter temperature during the irradiation processes of low-temperature product, and studies in the literature describing dosimeter response are performed under controlled conditions of fixed irradiation temperature and dose rate. Both observations make it far from straightforward to take into account the effects of low temperatures on dosimeter response during PQ dose-mapping of frozen product. Therefore, the preferred method for PQ dose-mapping of frozen serum is to conduct the study at ambient temperature conditions. Appropriate simulated product and/or simulated dry ice (discussed in section 3) is used with the dosimetry system, and calibrated for use by the operator of the gamma irradiation facility for typical sterilization processes.

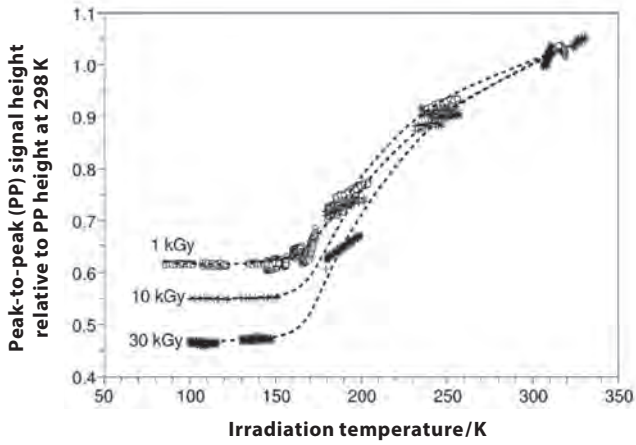

FIGURE 1. Relative response of alanine dosimeters irradiated to 1, 10, and 30 kGy at constant temperatures between 80 and 310 K (approximately –193°C to 37°C).[10] Dry ice has a temperature of 195 K (–78°C).

Gamma Irradiator

The various components of a typical gamma irradiator (**Figure 2**) and their functions were discussed in a previous article in this series.[1] The irradiator must have undergone

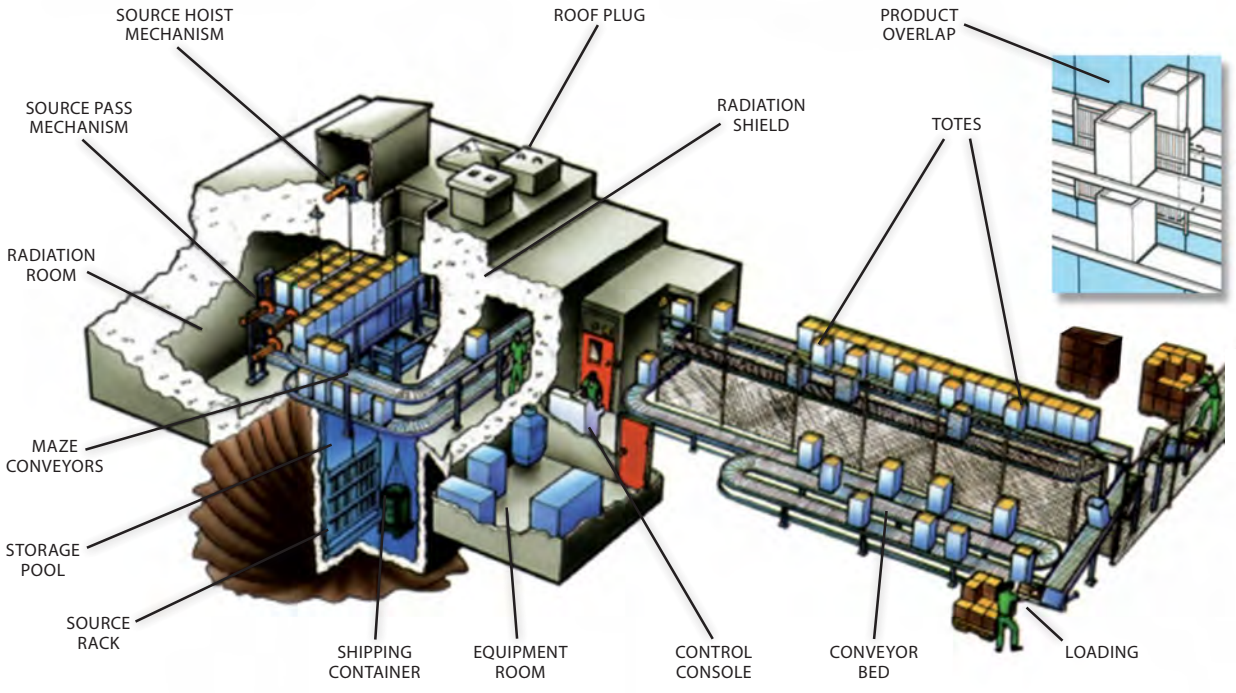

FIGURE 2. Schematic of a continuous (tote) irradiator. (Courtesy of MDS Nordion, Canada.)

installation qualification/operational qualification (IQ/OQ) for the pathway that the serum product will take through the irradiator.[3] Any irradiation facility that operated prior to the publication of the first version of ISO 11137 in 1995 may not have records of modifications to the facility undertaken during installation. Retrospective generation of such records is not required.

Each gamma irradiator has specific design specifications based on the business needs of the operating company. The specifications are verified as having been met by the execution of an established IQ process.

As a part of the qualification program, an irradiator needs to be characterized with respect to the magnitude, distribution, and reproducibility of dose delivery. Such a test program is called OQ. It is a prerequisite of OQ that all equipment which might have a critical effect on any of the dose delivery characteristics be maintained within specified limits. The main objective of OQ is to establish the facility's range of operating conditions and to provide baseline data for:

- Providing an understanding of the dose delivery characteristics (dose magnitude and distribution) for routine operation of the irradiator.
- Demonstrating reproducibility of the dose delivery process.
- Defining dosimeter positions for dose-mapping of product during PQ and re-qualification of the irradiator. (Guidance on OQ of changes to a gamma irradiation facility is provided in ISO 11137.[3])

OQ is achieved through a series of dose-mapping studies in which dosimeters are placed in a process load of material that is of homogeneous density and that completely fills the irradiation container. Such studies are performed using standard operating conditions. In addition, dose-mapping studies are undertaken at or beyond the standard operating conditions of the irradiation facility to demonstrate the limits of reliable performance.

Product and Process Definition

The gamma irradiation process to be qualified is specific to a certain product, and therefore the following information must be specified for the PQ study:

- The product, its packaging system, and any variations. The packaging system might include product containers (*e.g.,* bottles), plastic bags, cardboard boxes, and cryotainers.
- Established minimum and maximum acceptable product dose specifications to be used with the frozen serum.
- The manner of cold chain management throughout the irradiation process—cryotainers and proper placement of dry ice (position, quantity, and time) used to ensure that a cold chain is always maintained.

3. PQ Dose-Mapping Methodology

The protocol or procedure for the PQ dose-map study will have detailed sections describing the methods to be used. These will include the following aspects:

- Definition of the product load configuration(s) in the irradiation container.
- Description of the actual or simulated product for PQ dose-mapping, including material density, dimensions, and all other pertinent information.
- Dosimeter positions.
- Definition of the irradiation process for PQ dose-mapping.

Definition of Product Load Configuration in the Irradiation Container

Irradiation of frozen serum is typically performed in a load configuration that minimizes the depth of the serum in the direction perpendicular to the cobalt-60 source racks, and with the irradiation containers loaded to less than the defined maximum design capacity. Serum product is typically placed only in the part of the irradiation container where the radiation field is most uniform, which tends to be near its vertical center. A description of the materials used for, and the method of securing the product near the vertical center of the irradiation container, must be included in the process specification.

Where different serum product/product presentations are submitted for irradiation (*e.g.*, different bottle volumes, the same bottle volume but different bottle shape), processing categories can be defined based on product-related variables that affect the dose delivered to the product. Different products of the same processing category can be processed together based on the same PQ dose-mapping. The PQ mapping is then to be performed under conditions that are documented to be the extreme cases, from a dose delivery perspective. It should be noted that such a grouping strategy usually comes at the expense of a widening of the dose range that can be delivered to the product on a routine basis with the defined irradiation process.

If partially filled irradiation containers are present during routine product irradiation, their effect on dose delivery should be taken into account during PQ dose-mapping. This may be done, for instance, by mapping documented extreme conditions from a dose delivery perspective. Dedicated studies in the irradiator's OQ program (described in section 2) may not be appropriate for this purpose because of the non-homogeneous nature of the serum product and the homogeneous materials used for OQ dose-mapping. For routine irradiation, simulated product can be used in order to compensate for the absence of product and as such, to avoid partially filled irradiation containers. A description of the simulated product to be used for such purposes must be included in the process specification.

If product can move around in the cryotainer and/or

irradiation container during irradiation, this also could influence dose delivery characteristics. For instance, when serum bottles are not placed in a cardboard box, but directly in a dry ice bath, the bottles may move when the dry ice sublimates. The possibility of this occurring during routine product irradiation should be taken into account during PQ dose-mapping. This may be accomplished by mapping documented extreme conditions from a dose delivery perspective, or by limiting bottle movement with spacers. A description of the materials used for it, and the method of securing the product, must be included in the process specification.

Dry ice should be used during routine irradiation in order to maintain the cold chain. Because dry ice sublimates, the amount of dry ice present throughout the irradiation process is a variable that cannot be controlled precisely during routine irradiation. Therefore, the effect of dry ice in different quantities on dose delivery should be taken into account during PQ dose-mapping (*e.g.*, by mapping documented extreme conditions that can occur during routine irradiation).

Selection of Materials for Simulated Product and Dry Ice

As described in section 2 above, the general recommendation is to perform PQ dose-mapping under ambient conditions using appropriate materials which simulate product and/or dry ice. Possible candidates having a density and shape within the product container that are equivalent to frozen serum may be saline, water, agar gel, wax, or vegetable oil. Granulated sugar, water softener salt pellets, or animal feed pellets are examples of materials that might be used to simulate dry ice for PQ dose-mapping.

Dosimeter Positions

Dosimeters should be placed under a three-dimensional coordinate system, directly on the filled parts of the bottles, and possibly also within selected bottles in order to properly estimate dose extremes in the defined product load configuration. Care needs to be taken to assure that the environment does not affect dosimeter response to ionizing radiation. For instance, placing a dosimeter inside a bottle of saline solution might affect the dosimeter's response to ionizing radiation if it is not sufficiently protected from direct contact with the saline solution. Dosimeters may be packaged so they are not affected by environmental changes in humidity, but are not intended to contact liquids. The dosimeter manufacturer should be consulted for specific recommendations regarding use in a liquid environment. The position(s) for routine process monitoring should be included in the dosimeter positions for PQ dose-mapping. The position(s) defined for that purpose should be at ambient temperature during routine irradiation—for reasons discussed in section 2—and should move together with the product through the irradiator.

Irradiation Process

A minimum of three irradiation containers should be mapped during the PQ dose-mapping study. Irradiation containers for PQ dose-mapping can be irradiated consecutively or spread out in time or space in order to provide a more robust estimate of process variability, just as it will occur during commercial irradiation of frozen batches of serum. No changes in cycle time or conveyor speed should be made when the irradiation containers for PQ dose-mapping are in the irradiator.

Additional replicate PQ dose-map studies may be completed to gain a further understanding of the irradiation process for the serum, and can aid in estimating process variability and defining process target doses (particularly if there is not a solid baseline for irradiation of serum product in the selected irradiator). Narrow dose window requirements and the other specific challenges for frozen serum described above might require irradiation of product in dedicated processing categories. Possibly also specified material and loading configurations of these materials, effectively mimicking the serum product, would have to be used for a defined number of irradiation containers preceding and following the serum product for PQ dose mapping and routine irradiation in order to ensure consistent dose distribution and magnitude throughout the run.

4. Analysis and Documentation of PQ Dose-Mapping

Analysis of PQ dose-mapping data must include, at a minimum:

- Dose distribution characterization.
- An estimate of process variability, which will be refined based on routine process monitoring.
- An assessment of the capability of the irradiation process to routinely meet the specified product dose window.
- Calculation of ratio factors to build the experimental relationships between dose at the defined point of reference for routine process monitoring, and the minimum and maximum product dose achieved in the monitored irradiation container.

A summary report addressing the items listed in sections 2 and 3 above should be prepared and approved by the operator of the gamma irradiation facility and the company that is responsible for the release of the irradiated serum. On the basis of information generated in the PQ dose-mapping study and documented in the summary report, a process specification must be prepared for the routine irradiation of frozen serum.[3] The routine product irradiation process does not have to occur at the cycle time or conveyor speed (corrected for source decay) used for PQ dose-mapping, provided it can be demonstrated that there is no change in dose distribution.

The process specification should be developed by the

company responsible for the release of the irradiated serum in conjunction with the irradiation facility, and may include the following information:

- The product, its packaging system, and any variations. The packaging system might include product containers (*e.g.,* bottles), plastic bags, cardboard boxes, and cryotainers.
- Established minimum and maximum acceptable product dose specifications to be used with the frozen serum.
- The manner of cold chain management throughout the irradiation process—cryotainers and proper placement of dry ice (position, quantity, and time) used to ensure that a cold chain is always maintained.
- Product loading configuration(s) in the irradiation container. This should include, if applicable, a description of the materials and method used for securing the product, and a description of the simulated product used for completing partially filled irradiation containers.
- Pathway through the irradiator.
- Frequency and location of dosimeters used for routine monitoring, as well as the relationship between the dose measured at such positions and the dose delivered to the serum inside the monitored irradiation container.
- Description of other types of product that can be irradiated together with the serum product, if any.

Conclusions

This paper is the third in a series of articles intended to provide increased transparency around the processes involved in the gamma irradiation of frozen animal serum. The topical series was introduced in a separate paper in this journal.[1] The second paper in the series[2] describes the best practices for validating the efficacy of ionizing radiation for inactivating pathogens of interest, and for evaluating the potential effects of this radiation on the functional properties of the serum that is being irradiated. Such studies lead to the definition of a minimum required and a maximum allowed dose for irradiation of serum.

This paper has described the best practices used to qualify a process suitable for the routine gamma irradiation of frozen animal serum within defined limits. This qualification involves studies referred to as PQ dose-mapping. PQ dose-mapping is performed in the irradiator where the product will be processed, and the irradiation process employed in the PQ study should match, as closely as possible, the process expected to be routinely used for the gamma irradiation of the serum. The preferred process for PQ dose-mapping of frozen serum is conducted at ambient temperature using appropriate simulated product and/or simulated dry ice material with the dosimetry system as it is calibrated for use by the gamma irradiation facility operator in a typical sterilization process.

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Note

This series of papers is being authored with ISIA's support for the purpose of establishing best practices for processes employed in the gamma irradiation of animal serum for pathogen reduction. A dedicated group of contributing authors include serum suppliers, irradiators, customers (end-users), expert scientists, and authorities involved in regulatory matters.

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