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Gamma Irradiation of Animal Serum: General Regulatory Environment and Process Controls

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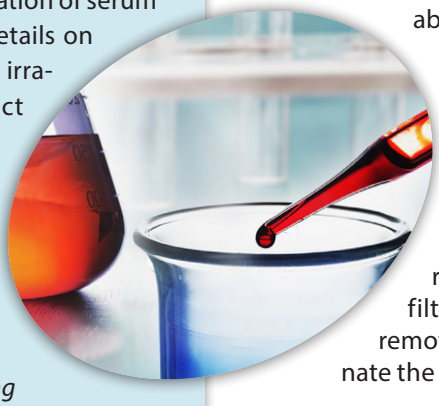
Abstract

This is the sixth and last in a series of articles describing and demystifying the processes involved in the gamma irradiation of serum. This serum treatment is intended to mitigate the risk of introducing adventitious contaminants into cell cultures. In this article, we discuss the regulatory environment under which gamma irradiation of serum is performed, and provide additional details on best practices for documentation of the irradiation process, selection of the contract irradiator, evaluation of risk versus benefit needed to arrive at the radiation dose range to be used, as well as an understanding of the level of remaining risk following irradiation at that dose range. Gamma irradiation should not be viewed as a means of totally *eliminating* risk, but rather as a means of *reducing* the risk of introducing adventitious agents into cell cultures. A balance must be achieved between the desire to eliminate all adventitious contaminants, and the need to retain the desired performance characteristics of the serum, once irradiated.



1. Introduction

This article is part of a series of papers that have been 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. These articles have been prepared and reviewed by a group of subject matter experts, serum users, irradiators, and serum suppliers (**Table 1**).

2. Background

Since the early days of cell culture, contamination by a variety of adventitious agents such as bacteria (including mycoplasma and mycobacteria), fungi, and viruses has been a concern.[1] Approaches for mitigating the risks of experiencing such contaminants have included manipulating cultures within laminar-flow hoods, practicing aseptic techniques, implementing the use of antibiotics (though not advised on a routine basis), and the screening and barrier treatment of cell culture reagents. Barrier treatments typically have included 0.1–0.45 µm filtration, heat inactivation, and irradiation by ultraviolet light, electron beam, or gamma photons. Animal serum such as fetal bovine serum (FBS) is sometimes required for successful expansion of a cell culture, and as stated above, the use of this animal-derived material carries the risk of bringing an adventitious agent into the culture. Manufactured lots of serum may contain some level of mycoplasma or virus contamination that is non-homogenously distributed between the many bottles comprising the lot. Mycoplasma and viruses are typically not removed by standard (0.2–0.45 µm) sterilizing filters. Triple 0.1 µm filtration is more likely to remove mycoplasma effectively, but will not eliminate the smaller viruses. Over the years, a particularly

TABLE 1. Gamma irradiation task force participants.

Participant	Affiliation	Representing
Sue Brown	TCS Biosciences	Supplier
Bart Croonenborghs	Sterigenics	Contract Irradiator
James Dunster	Moregate BioTech	Supplier
Debbie Elms	Thermo Fisher Scientific	Supplier
Randy Fitzgerald	RCC Consulting LLC	Supplier
Greg Hanson	GE Healthcare/HyClone	Supplier
Karl Hemmerich	Ageless Processing Technologies	End-user
Huw Hughes	Echo Veterinary Consulting	End-user
Robert J. Klostermann	Boehringer Ingelheim	End-user
Raymond Nims	RMC Pharmaceutical Solutions	End-user
Mark Plavsic	Lysogene	End-user
Mara Senescu	Medline Industries Inc.	Contract Irradiator
Marjorie van Robays	GlaxoSmithKline	End-user
Rosemary Versteegen	ISIA	Industry
Martell Winters	Nelson Laboratories	End-user

pervasive viral contaminant of FBS has been bovine viral diarrhea virus[2-3], a 40–70 nm enveloped virus that will easily pass through a 0.1 µm filter. Regulators have noted the problem and have issued guidance on serum manufacture and usage, as discussed below. A review of possible FBS treatment methods has been detailed previously.[4]

3. Examples of Adventitious Agents in Final Product

The European Economic Community (EEC) regulatory document: *Note for guidance: validation of virus removal and inactivation procedures*[5] states:

> "In the past, a number of biologicals administered to humans have been contaminated with viruses, and in several instances the contaminant was identified many years after the product had been introduced into the market."

In fact, there have been very few reported cases where a bovine viral contaminant has made its way into a biological product. These cases have primarily involved bovine viral diarrhea virus in veterinary vaccines.[6] However, there have been a number of reported cases of viral contaminants detected in biological bulk harvest samples. The viral contaminants attributed to the use of FBS have included epizootic hemorrhagic disease virus, Cache Valley virus, reovirus type 2, and vesivirus 2117.[7] When such viral contamination events occur, the bulk harvest may not be further processed and must be destroyed. It should be mentioned that gamma irradiation is known to be effective in inactivating all of the various viruses listed above.[7] To our knowledge, there has not been a reported biological contamination of final human product that can be attributed to the use of gamma-irradiated bovine serum.

4. Examples of Guidance and Regulations Relevant to Gamma Irradiation of Serum

One of the early regulatory guidance documents encouraging risk mitigation (barrier) treatment was the 1992 Balai Directive.[8] Chapter 7, *Blood and blood products of ungulates and poultry*, lists barrier treatments as an alternative to geographic safety and testing requirements (see Section 5 in this paper). Regulations related to the Balai Directive: (EC) No 1774/2002[9], (EC) No 1069/2009[10], (EC) No 142/2011[11], and (EC) No 294/2013[12] also discuss gamma irradiation at 25 kilograys (kGy) followed by an effectiveness check for blood products derived from Artiodactyla, Perissodactyla, and Proboscidea (ungulates and elephants), including their cross-breeds:

> "…guaranteeing the absence of pathogens of foot-and-mouth disease, vesicular stomatitis, rinderpest, peste des petits ruminants, Rift Valley fever and bluetongue."

The European guidance document, EMA/CHMP/BWP/457920/2012 rev 1[13], addresses the use of bovine serum in the manufacture of human biological medicinal products. The risk of viral contamination associated with the use of bovine serum is discussed, and it is stated that:

> "…it is strongly recommended, in addition to direct testing for viruses, to inactivate the serum by a validated and efficacious treatment. The use of non-inactivated serum should be justified."

The guidance document further states that:

> "Gamma irradiation is the most commonly used method for viral inactivation of serum as a means of obtaining a safe but biologically active product."

It also indicates that, when inactivating by gamma irradiation, the validation of the irradiation process should include the determination of the optimal temperature, establishment of a standard packaging configuration, dose mapping, and determination of a dose range that protects product integrity while maximizing contaminant inactivation.

The European Pharmacopoeia monograph 2262, entitled *Bovine Serum*[14], states that:

> "The inactivation procedure applied is validated with respect to a suitable representative range of viruses covering different types (enveloped, non-enveloped, DNA, RNA viruses). The optimal choice of relevant and model viruses depends strongly on the specific inactivation/removal procedure; representative viruses with different degrees of resistance to the type of treatment must be included. Bovine viral diarrhoea virus must be included in the viruses used for validation. Serum free from antibodies against bovine viral diarrhea virus must be used in part or all of the validation studies. For bovine serum intended for use in immunological veterinary medicinal products, inactivation by gamma irradiation at a minimum dose of 30 kGy is to be applied, unless otherwise justified and authorized."[14]

The monograph also discusses the critical parameters of the gamma irradiation process validation.

United States Pharmacopeia (USP) Chapter <1024>, entitled *Bovine Serum*[15], states that:

> "Serum treatment by gamma irradiation is very common and one of the most effective methods of virus inactivation. The most frequently used minimum dose is 25 kiloGrays (kGy)."

The USP chapter also indicates that some countries specify higher irradiation doses for imported serum, but warns that users should ensure the dose applied will not negatively affect the specific serum application that is intended. USP <1024> also discusses various aspects of the validation of gamma irradiation. USP <90>, entitled *Fetal Bovine Serum—Quality Attributes and Functionality Tests*[16] includes the statements:

> "…gamma irradiation provides the highest assurance of the absence of viral activity. Gamma irradiation doses of 25–40 kGy provide significant log reduction of viral and other adventitious agents while preserving cellular growth performance".[16]

USP <1043>, entitled *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products*[17], states:

> "…FBS can be obtained that has been processed to reduce the risk of bovine viral contamination by subjecting it to validated irradiation and nanofiltration processes."

In *Gamma irradiation as a treatment to address pathogens of animal biosecurity concern* (Australian Department of Agriculture)[18], 50 kGy is the current standard for importation of serum into Australia and New Zealand, though if justified, this dose may be decreased. If the importer can determine that only viruses that are highly susceptible to ionizing radiation are present in the product, then an argument may be presented for reducing the 50 kGy threshold. It must be noted that significant loss of serum functionality may occur at doses ≥50 kGy.

5. Proposed Barrier Treatments for Mitigating Adventitious Agent Risk in Serum

The Balai Directive[8] and subsequent legislation[9-12] suggest that blood products be subjected to one of the following treatments:

- Heat treatment at a temperature of 65°C for at least three hours, followed by an effectiveness check
- Irradiation at 2.5 megarads (25 kGy), followed by an effectiveness check
- Change in pH to pH 5 for two hours, followed by an effectiveness check
- Heat treatment of at least 90°C throughout their substance, followed by an effectiveness check

As previously discussed, gamma irradiation of serum in the final containers, in standard load configurations, while deeply frozen is the least damaging and most effective of these recommended barrier treatments[4], although it must be noted that irradiation of serum may affect undefined factors in the serum that promote cell growth, apparently in a dose-dependent manner.[19-22] In conclusion, it is clear that regulators worldwide have noted the efficacy of gamma irradiation for the inactivation of viral adventitious agents in serum for cell culture applications. It is also important to realize that at the doses normally applied, not all families of viruses will be inactivated. In particular, parvoviruses, circoviruses, and polyomaviruses may survive this treatment.[21]

6. Risk/Benefit Analysis

The decision of whether to have serum irradiated, and the appropriate radiation dose to use must be made by the final user (*e.g.*, the biological product manufacturer, research lab, *etc.*). Studies may be carried out to assess the effects that irradiation of serum has on the specific serum application of the user.[21, 22] Such assessments might include evaluation of cell growth, product yield and quality, and production time, which affects manufacturing cost of goods. These data may then be used in a risk/benefit analysis in which the risk of product contamination, and therefore loss, may be weighed against the risk of lower production yield and/or increased cost of goods. In a high-volume production environment, irradiated vs. non-irradiated serum comparability testing might not be easily demonstrated. Thus, it is a good idea to use irradiated serum from the earliest stages of product development.

The use of non-irradiated serum or serum irradiated at the lower end of the typical range of 25–40 kGy may require the manufacturer to complete lot-by-lot bovine virus testing during the manufacture of a product. The additional cost and time required for this testing should be taken into account when considering risks/benefits. As mentioned previously, most regulatory agencies have adopted a low tolerance for what they consider the unnecessary use of non-treated animal-derived products. The failure to treat such raw materials must be justified. In addition, some jurisdictions (*e.g.*, Australia and New Zealand) may require complete extraneous agent testing of materials to be imported, regardless of the dose of irradiation used. Different regions or countries recommend/require different doses of gamma irradiation in order to be compliant. As discussed above, the European Union requires 30 kGy for veterinary biological products.[14] The Australian Final Policy Review states that 50 kGy remains the current standard, with increased doses recommended in certain cases.[18]

The assessed patient risk of contamination in a manufacturing process by adventitious agents also depends on the type of biological product (inactivated vs. live vaccines, for instance), the patient profile (healthy vs. diseased), and the geographical origin of the raw materials used. When considering risk, the complete biological production process must be taken into consideration, as adventitious agent contaminants may be resistant to even the highest doses of irradiation used.[21] A guideline for conducting such a risk assessment can be found in European Pharmacopoeia Chapter 5.1.7[23], entitled *Viral Safety* (see also [24]). Risk assessment therefore is a complex process for biological products that must take into account the necessity of additional testing of the serum and bulk harvest, the country or regions of serum origin, the countries or regions in which the products are to be sold, effects that irradiation of serum may have on the manufacturing process and product, and the risk/benefit considerations of the product for the patient.

Another aspect of risk that should be considered relates to the point in the serum production process when irradiation should be performed. While bulk material can be irradiated, the material must then be handled extensively through pooling, aseptic filling, packaging and labelling. Such handling increases the chance of contamination. It is therefore strongly recommended that irradiation be performed in the final filled and labelled serum bottle.

7. Control of the Irradiation Process

Product and irradiation process specifications should be developed from information generated during process definition (*i.e.*, establishing minimum required and maximum acceptable radiation doses for the serum product)[21] and performance qualification (PQ) dose map studies.[25] Process specifications should also clearly define all requirements, including those for maintaining the cold chain.[26] **Figure 1** demonstrates the complexity of the serum irradiation process.

Specific requirements for irradiation should be included in

a process specification, which must be approved by both the provider of the irradiation service and the customer requesting the irradiation (serum supplier or end-user). Examples of process specification items to consider are shown in **Table 2.**

Procedures then have to be in place for the irradiation process to be shown to be in a state of control. Routine monitoring and control of the irradiation process confirms that the specific activities needed to ensure process dose requirements are achieved have been performed. Process controls during routine irradiation have been detailed previously.[25]

The dose delivered to the serum product is monitored using a dosimetry system which has been calibrated for the conditions of use and is traceable to national or international standards. Upon completion of the irradiation process, the processing history records must be reviewed and approved by designated contract irradiator personnel. Processing history records for irradiation of frozen serum typically include the items shown in **Table 3**.

Given the importance of dosage, as described above and in other publications[4, 17, 19], a statement of the radiation dose range delivered is required by the customer. On the basis of approved processing history records, the contract irradiator will issue a certificate of irradiation (CoI). The CoI provides formal evidence of the radiation dose range delivered during the

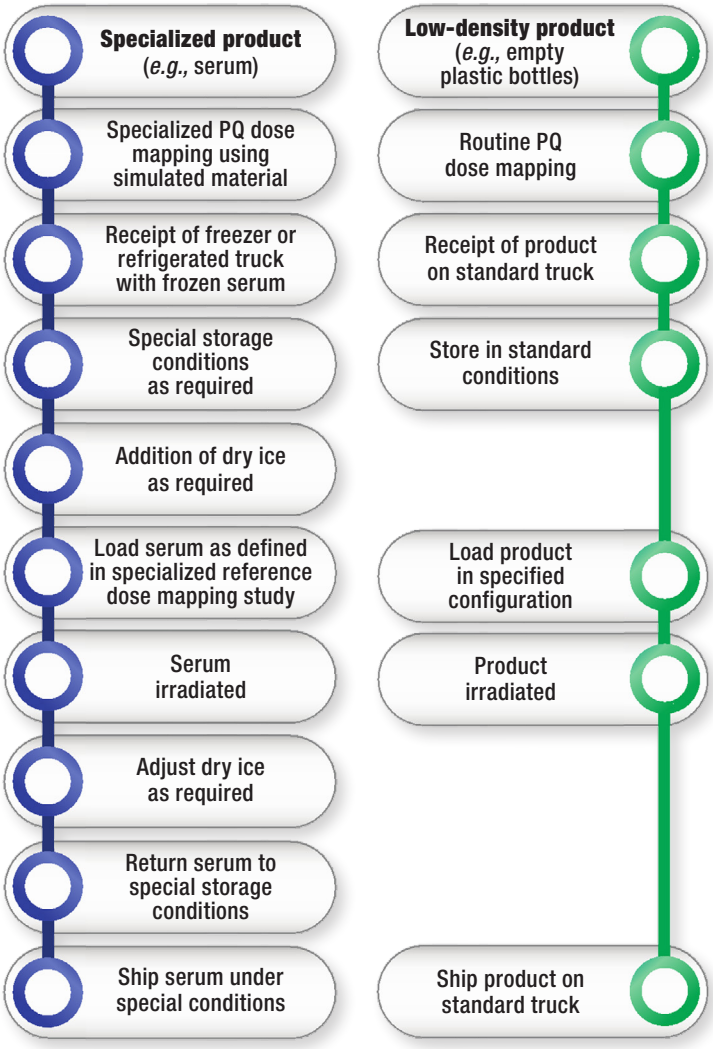


FIGURE 1. Comparison of process flow for irradiation of serum vs. irradiation of low-density materials. PQ

TABLE 2. Items to consider in process specification.

Step	Details
1	Description of the packaged serum product, including dimensions, density, and orientation of product within the packaging system(s), and acceptable variations.
2	Maximum acceptable radiation dose for the serum product (the dose at which it is still expected to meet all functional specifications)
3	Minimum required radiation dose for achieving the specified reduction of microbial contaminants that might be present in the serum product before irradiation.
4	Minimum and maximum specifications of the dose to be delivered at the routine monitoring position.
5	Loading pattern(s) of serum product within the irradiation container.
6	Pathway(s) through the irradiator that will be used.
7	Measures for maintaining the cold chain during storage, as well as during irradiation. This should include specifics about the use of dry ice (position, quantity, and time of addition).
8	Suitable cycle timer settings, and details of other products or materials that can be irradiated concurrently with the serum.
9	Routine dosimeter monitoring position(s) and placement frequency.
10	Relationships between the radiation dose at the monitoring position(s) and the minimum and maximum doses for the serum in the monitored irradiation container.

TABLE 3. Gamma irradiation history record requirements.

Step	Details
1	Serum receiving records.
2	Product count verification, records of discrepancies, and actions taken (if applicable).
3	Irradiation container loading and unloading records.
4	Processing records (cycle timer setting, processing schedule information, interruptions of the irradiation process).
5	Conveyor operation and/or pathways.
6	Processing or storage deviations, and associated investigations and corrective actions (if applicable).
7	Dosimeter measurements at the routine monitoring position, and/or calculated minimum and maximum doses for serum product in the monitored irradiation containers. These values then should be compared with the values documented in the processing specification.

irradiation run, including values for minimum and maximum calculated doses received by the serum. If these calculated doses are within the range required by the customer, the batch of serum identified on the CoI has been irradiated satisfactorily. As previously discussed, the dose of irradiation must meet the regulatory requirements for both the country of origin and the country/region of intended use. These requirements, as well as those for product movement, are country-dependent and should be verified by consultation with local authorities.

Processing data can be accumulated, organized, and reviewed to evaluate the consistency of the irradiation process over time. This data may include, for example, results of dose measurements, processing parameters, and non-conformances. The results can be used to identify processing runs that have approached the processing specification limit(s) and possible process trends, allowing for corrective actions to be taken.

The information and requirements needed for the documentation of the irradiation process results are different from the information and requirements required by the end-user. The end-user will typically expect to receive a certificate of analysis (CoA) that states compliance with current regulations, as well as other information relevant to the use of the product. In some cases, the contract irradiator's customer is the actual end-user. **Table 4** lists information that should be part of the CoA given to the end-user. In addition, information on leachables and extractables that might be detected following irradiation (especially for higher doses in non-glass containers[28]) should be available to the end-user upon request.

TABLE 4. Information to be shown on a serum product CoA.

Step	Details
1	Country of origin of the serum.
2	A description of the packaged serum product, including type of container (*e.g.*, HDPE, glass, *etc.*), volume, and acceptable variations.
3	The maximum acceptable radiation dose for the serum product (*i.e.*, dose at which the serum still meets all of its claimed functional specifications and methods by which function was assessed [*e.g.*, cell growth]).
4	The minimum required dose defined by Pharmacopeia[14,15] or equivalent for achieving the specified reduction of defined organisms that might be present in the serum product before irradiation.
5	Guarantee of cold chain (below –15°C to –20°C recommended) from the first freeze to the time of shipment of the irradiated serum to the end-user.
6	ISIA Traceability Certification of the serum (recommended).
7	Other items typically included on a serum CoA, such as physicochemical characteristics (density, opacity, color), biochemistry, *etc.*, and extraneous agent tests and methods of testing (*e.g.*, European Pharmacopoeia[15], 9 CFR 113.47[27], *etc.*).

8. Points to Consider in the Selection of a Contract Irradiator

There are many points to consider when the serum supplier chooses a contract irradiator, as they need to consider the exacting needs of their customers (the end-users). Serum irradiation is far more complex and time-intensive than the irradiation of low-density products (such as plastic serum containers) for sterilization purposes (**Figure 1**). Capacity and lead time at gamma irradiators can depend on the particular process requirements and the scheduling calendar (*e.g.*, most irradiators don't work weekends). Gamma irradiation dosing requirements are very exacting when working with frozen serum and dry ice.

Advanced planning for irradiation of serum may be difficult. Typically, end-users conduct some testing of different serum lots prior to acceptance. If the accepted lot is to be irradiated, the request for irradiation is made following supplier notification of client acceptance. The complexity of the irradiation process means that turnaround times may be difficult to obtain from the irradiator. In addition, if the contract irradiator is not able to ship irradiated serum directly to the end-user, the supplier will need to arrange pickup of the material post-irradiation, and then must schedule onward shipment to the end-user. Irradiator capabilities can vary widely, as one may be able to offer a specific dose range, but another cannot due to the use of different equipment. It must be noted that narrow dose ranges are substantially more difficult to achieve and hence, more costly.[25]

A well-informed supplier working with a skilled contract irradiator will be able to resolve many of the issues that have been highlighted in this review of serum irradiation. The end-user must also be educated in the irradiation process and understand that their suppliers must rely on a number of vendors to deliver their irradiated serum. Third parties include transport companies, contract irradiators, and more. It takes a well-coordinated and highly scheduled effort to successfully deliver accurately irradiated serum.

9. Conclusion

All commercial lots of serum are likely to have some level of virus or mollicute contamination. Gamma irradiation is an accepted and recommended barrier treatment for lowering the risk of adventitious agent contamination associated with serum use in cell cultures. Regulatory authorities now expect justification for the use of bovine serum not subjected to a barrier treatment such as gamma irradiation. A balanced approach must be arrived at between the desire for effective inactivation of pathogens in serum (*i.e.*, higher irradiation doses) and the need to preserve serum performance (*i.e.*, lower irradiation doses). This dose range will vary between end-users on the basis of their specific culture application and the regulatory environment they operate in. The applied gamma irradiation dose must be documented on a CoI or certificate of processing provided by the contract irradiator. This allows the end-user to confidently accept the irradiated product for use in their biological process. Contract serum irradiation organizations should be selected on the basis of their ability to meet the needs of suppliers and end-users while ensuring the required irradiation dosing, product quality, consistency, and delivery of the material in the necessary time frame.

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Note

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