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Gamma Irradiation of Animal Serum: Maintaining the Cold Chain Throughout the Process

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Abstract

This paper reviews the importance of maintaining low temperature storage and handling (*i.e.*, cold chain) for animal serum through all stages of processing, from finished product to the actual end-user. This cold chain extends from serum manufacture through the irradiation process, during shipment back to the supplier post-irradiation, as well as storage at supplier, irradiation, and end-user facilities. Anecdotal experience and theoretical considerations emphasize the point that maintenance of the cold chain is necessary for preserving the performance of serum for cell culture and other applications.

Introduction

This article is part of a series of papers that are being authored under the sponsorship of the International Serum Industry Association (ISIA)[1] with the purpose of establishing best practices for processes employed in the gamma irradiation of animal serum. Gamma irradiation is performed for the purpose of pathogen (especially virus and mollicute) reduction, as mandated by various regulatory bodies and pharmacopeial organizations.[2-5] In order to preserve and maximize the concentrations and activities of serum nutrients and growth factors required for cell culture medium supplementation and other applications, it is necessary to maintain serum at a low temperature prior to use. Freezing and thawing of serum has been shown[6] to negatively affect its biological activity, as a result of the degradation of critical components. In the present article, we describe the importance and means of controlling the temperature of bovine serum throughout the entire process of gamma irradiation. This includes not only the actual irradiation process, but also the shipment of serum to and from the irradiation facility, and any intervening periods of storage. In fact, both the United States Pharmacopeia and European Pharmacopoeia chapters addressing fetal bovine serum (USP <1024>[4] and Ph. Eur. 01/2008:2262[5], respectively) cite temperature control as a critical parameter in gamma irradiation. Both documents also state that serum must be routinely stored frozen at or below –10°C.

As previously discussed in this series[1,7], the mechanisms underlying both the desired microbial inactivation and undesired adverse effects on serum components by gamma irradiation are highly temperature-dependent. At higher temperatures (especially above freezing) and in the presence of oxygen, the production and diffusion of oxygen radicals predominates. Under these conditions, some degradation of serum components is to be expected. At very low temperatures in sealed containers, the generation and diffusion of oxygen radicals is minimized, while the direct effects of gamma radiation on microbial genomic material are favored. The efficacy of gamma irradiation for microbial inactivation at the typical deeply frozen state has been discussed previously, as has been the methods that are used for assessing the effects of gamma irradiation on serum performance.[7] The optimization of irradiation temperature for preservation of serum performance can be achieved through empirical studies in which the experiments properly control the delivered radiation dose as well as the irradiation temperature. Unfortunately, such well-controlled studies have not been described in the literature. In the present paper, we describe the current best practices for temperature control of animal serum used throughout the entire irradiation cold chain.

The Gamma Irradiation Cold Chain

As mentioned above, maintenance and documentation of the cold chain is an essential part of the serum irradiation process. The cold chain extends from manufacture of the serum, through the irradiation process itself, and during shipment back to the supplier post-irradiation (**Figure 1**). Note that the typical target temperatures associated with different steps of the cold chain may differ. For instance, during the irradiation process, it is not uncommon for dry ice to be used as the coolant, which would be expected to result in product (serum) temperatures much lower than the –10°C temperature stipulated in the compendia chapters mentioned above for storage.[4, 5]

Storage of the Serum Following Manufacture

Prior to sale of serum in the commercial market, and prior to shipment to irradiation facilities (in the case of serum intended for gamma irradiation), the product must be stored under proper conditions by the supplier. Typically, this means storage at or below –20°C (the typical set temperature for laboratory freezers) in non-defrosting freezers. During a defrost cycle, the temperature inside a freezer is briefly raised to eliminate or reduce frost, and such temperature fluctuations can be detrimental to serum.

Transportation of Serum To and From the Irradiation Facility

As mentioned previously in this series[1], serum suppliers do not irradiate serum. Gamma irradiation is performed by one of a relatively few irradiation facilities that are equipped for this purpose. They have the necessary expertise and have conducted dose-mapping studies to assure that intended gamma radiation fluencies (absorbed doses) will be achieved routinely during the irradiation process.[8] Serum to be gamma irradiated must therefore be shipped under appropriate conditions from the supplier to the irradiation facility and then back to the supplier following irradiation.

Ensuring that serum remains frozen during transport to and from an irradiation facility is typically achieved by the addition of dry ice to the shipping containers and/or the use of temperature-controlled transport vehicles. As dry ice is considered hazardous for transport (UN 1845) due to the generation of carbon dioxide gas, there are requirements associated with the air and ground transport of goods packed with dry ice, including:

- The package must be vented to allow for the release of carbon dioxide gas.
- The shipper must determine the appropriate amount of dry ice.
- If a corrugated box is used as the shipping container, it should be of a heavy gauge.
- Any void spaces in the shipping container should be filled with inert fill material.

Regulations for the shipment of dangerous goods in Europe (ADR)[9] require the use of appropriate signage on vehicles transporting goods with dry ice as a coolant. In the United States, local and federal Department of Transportation (DOT) regulations must be followed for shipping goods on dry ice.[10] In other countries, specific national regulations may also apply. Evidence of cold chain maintenance should be obtained during transportation of serum to the irradiation facility and back to the supplier. This may be achieved through the use of temperature monitors included in the shipping containers and/or through temperature logging data requested from the shipping contractor. It is particularly important to include temperature monitors with air shipments to assure that the cold chain is maintained over the course of extended flights with layovers. The temperature monitors used must be capable of recording temperatures down to –80°C (since the sublimation temperature of dry ice is –78.5°C).

Storage of Serum at the Irradiation Facility Before and After Irradiation

Planning for the irradiation of the serum should include the irradiation plant process (*i.e.*, the gamma irradiation itself) scheduling, availability of freezer space at the facility, and segregation of pre- and post-irradiation serum shipping containers. If there is insufficient freezer space at the irradiation facility, the use of a validated and monitored freezer truck sited as close to the facility as is practical

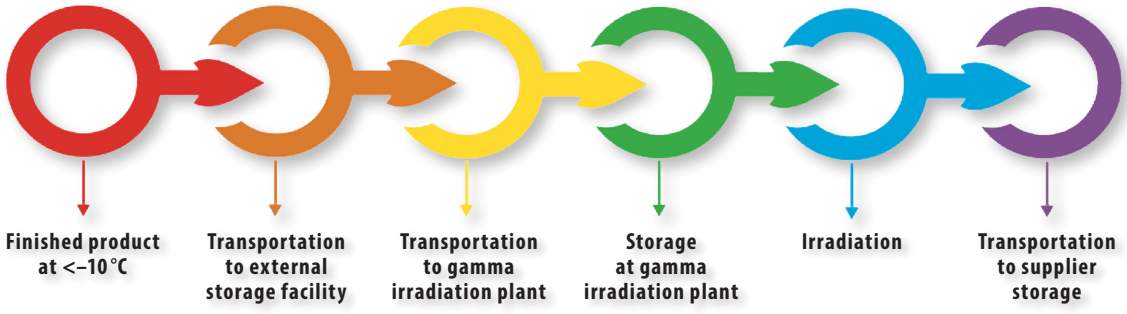

FIGURE 1. The cold chain for gamma irradiation of serum extends well beyond the irradiation process itself.

may represent a temporary storage solution. Removing and returning the serum shipping containers to the freezer or freezer truck at the irradiation facility must be done as efficiently as possible to minimize temperature fluctuations while ensuring segregation of irradiated and non-irradiated product. This requires consideration of the layout of the irradiation plant and staff availability during the processing run. It is advised that serum for irradiation be shipped to the irradiation facility relatively close to the scheduled processing time in order to minimize storage time at the irradiation facility.

Maintenance of Cold Chain During Gamma Irradiation

Generally, the temperature monitoring devices used during transport of serum to the irradiation facility will be removed and the temperature information downloaded once the shipment arrives. However, irradiation facilities may not have the particular software and/or equipment required to download the data. In these instances, the temperature recorders may be shipped back to the client, usually at client expense. Temperature monitors are not typically used during the gamma irradiation process since it is unlikely that digital or electronic temperature monitors could withstand the irradiation process, and temperature data might be erased. It is imperative that the serum remains frozen during the irradiation process, as adverse effects on serum performance might occur otherwise. As ionizing radiation is a form of energy, gamma dose delivery will inherently introduce heat to the serum. Depending on the activity of the cobalt source used and irradiator design, it is not uncommon for temperatures in the irradiator to reach as high as 50°C. Typically, the serum will be in the irradiator for several hours during which it will convey multiple times beside the unshielded source of radiation.[8]

In addition, consideration has to be given to handling time before and after irradiation as serum batches typically represent large process loads (typically 2000 L). The time and temperature associated with the processing of a given serum load is also influenced by the geographic location of the irradiation facility and seasonality (esp., winter vs. summer), as these determine the fluctuations in external ambient temperature. Packaging and packing requirements for irradiation (including the amount of dry ice needed and its potential effect on dose delivery) are considered as part of the performance qualification (PQ) of the irradiation process, and have been described previously in this series.[8] Batches of serum sent for irradiation must be packed in the same way as those for which the PQ dose mapping was carried out. Undertaking a temperature qualification study at the hottest time of year is one way to ensure that the amount of dry ice required for routine irradiation, and considered for PQ dose mapping, is being determined under worst-case conditions.

The addition of dry ice to serum shipping containers immediately prior to irradiation is done according to weight or volume determined during temperature validation studies — if applicable — and taken into account in the PQ dose-mapping validation. This information needs to be included in the routine processing instructions used at the irradiation facility. Since more than one pass through the irradiator may be necessary to achieve the required radiation dose, dry ice may need to be added between consecutive irradiations. The quantity of dry ice required and the sublimation rate are influenced by the shipping container type, how tightly the serum bottles are packed within the shipping container, and the location of the dry ice. If the dry ice is in direct thermal contact with the bottles, a temperature gradient may be established within the product, and the amount of radiation shielding conferred by the dry ice may be highly variable as the dry ice sublimates during the irradiation process. For these reasons, it is preferable (and the best practice) that a physical barrier be created between the dry ice and the serum bottles. This is true even if such a barrier means that the temperature of the serum may not be as low because of such separation.

If a cryotainer is provided by the irradiation facility, dry ice can be added to specific locations within the cryotainer in a manner ensuring that its presence does not influence dose delivery characteristics or create a temperature gradient within the product. The best practice for a given process run (*i.e.*, direct dry ice contact/lower temperature/temperature gradient vs. physical barrier to dry ice/higher temperature/no temperature gradient) must be considered before or as part of the dose-mapping validation.

For routine irradiation, records should be kept to document the quantities of dry ice added as well as when they were added. Evidence that serum remains frozen (below 0°C) for the duration of the irradiation can be obtained by a visual check that dry ice remains present following completion of irradiation. It is essential that the irradiator operator confirms that dry ice was present throughout the entire irradiation process. This is the only practical indication that the serum was maintained as deeply frozen during irradiation.

Can the Optimal Temperature for Irradiation be Empirically Determined?

As mentioned above and in previous articles in this series[1,7], the generation and diffusion of oxygen radicals in serum during gamma irradiation is significant at higher temperatures and in the presence of oxygen. The potential adverse effects of oxygen radicals on serum performance may be avoided by irradiating serum in the original sealed containers (typically plastic bottles) at temperatures well below freezing. Under such conditions, especially in the radical-scavenging milieu provided by bovine serum, oxygen radical generation and diffusion is limited, and the direct effects of ionizing radiation on microbial nucleic acids will prevail. Theoretical considerations therefore suggest that the lower the temperature for irradiation the better, but how may one empirically demonstrate this?

The design of a study to demonstrate the optimal

irradiation temperature for preservation of serum performance must allow for:

1) accurate measurement of the serum temperature ranges experienced during the irradiation;
2) accurate dose delivery and dosimetry such that the intended dose is achieved and does not vary significantly between the differing temperature ranges to be evaluated; and
3) a means of assessing serum performance before and following irradiation.

As mentioned previously in this series[8], it is difficult to adequately monitor dose and product temperature during irradiation processes of low temperature product, and studies in the referenced literature addressing the dose response have been performed under controlled conditions of fixed irradiation temperature and dose rate. Neither of these apply to a commercial irradiator. The typical dose that is targeted for pathogen reduction is 30–50 kilogray (kGy), and control of the dose to within a tighter range may be difficult and costly. Therefore, the dose achieved in any given empirical study is most accurately expressed as a range, and not a single value.

Serum performance can be assessed through a variety of biochemical and cell culture approaches.[7] Most commonly, comparisons are made for the growth or viability of various cell lines maintained in culture medium supplemented with the irradiated vs. non-irradiated serum. The most sensitive indicator of serum performance is its ability to support cell attachment and growth at low seeding concentrations (colony formation assay or plating efficiency assay). Comparisons may also be made for one or more serum enzyme activities, nutrient or hormone concentrations, or levels of total or specific proteins as determined by electrophoresis. A hypothetical example of the latter experiment is displayed in **Figure 2**. Non-irradiated fetal bovine serum exhibits a typical banding pattern that is altered to a greater extent when irradiated while non-frozen, and less perturbation is observed when irradiated while cooled with dry ice.

The main point of the discussion above is that controlling and varying both temperature and dose simultaneously is technically quite difficult, and to our knowledge, no well controlled studies of this type have been published to date. On the other hand, it should be re-emphasized here that the best serum irradiation practice is not to maintain a given temperature during irradiation, as this is not practically achievable, but rather to maintain a deeply frozen condition through the validated use of dry ice coolant in the shipping or irradiation containers (provided by the irradiation operator) used to convey the serum product through the irradiator.

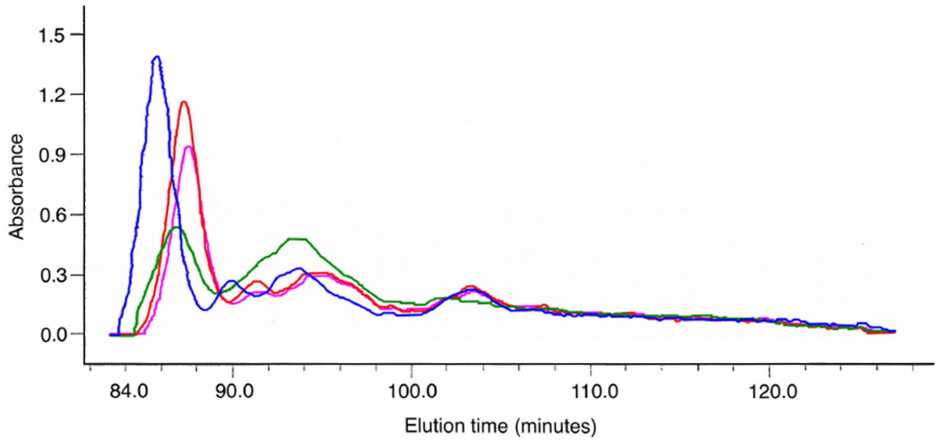

FIGURE 2. Serum banding patterns that might be expected on Sepraphore gels following electrophoresis. Blue trace: non-irradiated serum; green trace: serum irradiated at 2–8°C; red and magenta traces: serum irradiated while in dry ice.

Conclusions

The efficacy of gamma irradiation for the reduction of pathogen levels (especially viruses and mollicutes) has been reviewed previously.[7, 11] It is important to note that these studies have been conducted using serum that was gamma irradiated while deeply frozen. For years, the exact temperature at which gamma irradiation of serum has been conducted has been considered proprietary information. Therefore the reports of efficacy originating from serum suppliers have been a bit vague with respect to this important parameter. The importance associated with the irradiation temperature is not so much with regard to efficacy for pathogen inactivation as it is with the preservation of the desired properties of the serum. Irradiation at higher temperatures (*i.e.*, above freezing) and at higher doses (*i.e.*, > 50 kGy) might be expected to cause greater pathogen reduction, but almost certainly at the unacceptable expense of serum performance.

In this article, we discuss some of the difficulties associated with empirically determining, achieving, and recording an optimally defined temperature for the serum

irradiation process. Theoretical considerations indicate that the serum must be kept frozen during the entire irradiation process if diffusion of harmful reactive oxygen species is to be limited. The best practice among irradiators is to employ sufficient dry ice as coolant to maintain the serum in a frozen state throughout the irradiation process.

Other than the theoretical argument presented above, there have only been a few anecdotal reports of adverse effects to serum performance from the failure to maintain the cold chain throughout the transport and irradiation process. For instance, suppliers have noted that if the cold chain is not controlled, gross serum layering inside the primary container may be observed once the product is thawed. This happened to one irradiator in fewer than 10% of serum batches, which led to lot rejection and manufacturing cost increases when it did occur.[12] Gauvin and Nims[13] reported the occurrence of filterability issues with cell perfusion media compounded with gamma-irradiated serum. Variability in the temperature of the serum before, during, and after gamma-irradiation was subsequently determined to be the cause of the effects observed. The issues were resolved by improving temperature control during serum shipment to and from the irradiation facility, and during the gamma-irradiation process itself. This illustrates the importance, described in this paper, of maintaining the cold chain throughout the serum storage, transport, and irradiation process. A break at any point in the cold chain might lead to adverse effects on serum performance such as that described above.[12,13]

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References

[1] Versteegen R, Plavsic M, Nims R, Klostermann R, Hemmerich K. Gamma irradiation of animal serum: an introduction. *BioProcess J*, 2016; 15(2): 5–11. http://dx.doi.org/10.12665/J152.Versteegen

[2] European Medicines Agency. *Requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products.* EMEA/CVMP/743/00 rev. 2, 9 Nov 2005. http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500004575

[3] European Medicines Agency. *Guideline on the use of bovine serum in the manufacture of human biological medicinal products.* EMA/CHMP/BWP/457920/2012 rev. 1, 30 May 2013. http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500143930

[4] United States Pharmacopeia <1024>. *Bovine serum.*

[5] European Pharmacopoeia. *Bovine serum.* 01/2008:2262. http://online.pheur.org/en/entry.htm

[6] Transparency Market Research. *US biopharmaceutical market–global industry size, market share, smart trends, analysis and forecast 2012–2018.* http://www.transparencymarketresearch.com/us-biopharmaceutical-market.html

[7] Plavsic M, Nims R, Wintgens M, Versteegen R. Gamma irradiation of animal serum: validation of efficacy for pathogen reduction and assessment of impacts on serum performance. *BioProcess J*, 2016; 15(2): 12–21. http://dx.doi.org/10.12665/J152.Plavsic

[8] Croonenborghs B, Pratt A, Bone L, Senescu M. Gamma irradiation of frozen animal serum: dose mapping for irradiation process validation. *BioProcess J*, 2016; 15(3): 7–13. https://doi.org/10.12665/J153.Croonenborghs

[9] ADR – European agreement concerning the international carriage of dangerous goods by road. ECE/TRANS/257 volumes 1 and 2; 1 Jan 2017.

[10] United States Code of Federal Regulations 49 CFR 172.102 – Special provisions.

[11] Nims RW, Gauvin G, Plavsic M. Gamma irradiation of animal sera for inactivation of viruses and mollicutes – a review. *Biologicals* 2011; 39: 370-7.

[12] Plavsic M. Unpublished data.

[13] Gauvin G, Nims R. Gamma-irradiation of serum for the inactivation of adventitious contaminants. *PDA J Pharm Sci Technol*, 2010; 64: 432-5.

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