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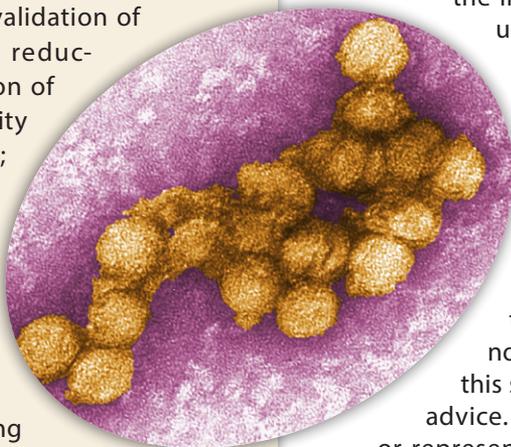
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Gamma Irradiation of Animal Serum: An Introduction

By Rosemary Versteegen, Mark Plavsic, Raymond Nims, Robert Klostermann, and Karl Hemmerich

Abstract

This article serves as an introduction to a series of papers that are being authored under the sponsorship of the International Serum Industry Association with the purpose of establishing best practices for processes employed in the gamma irradiation of animal serum. It is comprised of a discussion about the role of serum in cell culture and the management of the associated risks. Additional articles in the series will address a number of topics of interest to the cell culture community, including, but not limited to: (1) performance of absorbed dose mapping for irradiators; (2) validation of the efficacy of pathogen reduction during gamma irradiation of animal serum; (3) comparability evaluation of irradiated serum; (4) product management throughout the irradiation process; and (5) ensuring a quality outcome when using gamma irradiation. The intent of the series is to increase awareness of the scientific community regarding the conduct of gamma irradiation and the strengths and limitations of this serum treatment approach for achieving the goals of adventitious agent risk mitigation.



members of the Association provide greater than 90% of the animal serum and animal-derived products used in life science research, and biotherapeutic, vaccine, and *in vitro* diagnostic manufacturing. The ISIA recognizes the requirement for robust risk assessment and has several ongoing programs designed to help mitigate the risks associated with use of animal-derived materials. As part of this effort, ISIA strongly recommends post-manufacturing gamma irradiation of animal serum.

It should be noted that gamma irradiation sterilization of medical devices is a validated process (described in ANSI/AAMI/ISO 11137 Sterilization of Healthcare Products—Radiation).^[1] On the other hand, gamma irradiation treatment of serum products is a risk mitigation step for adventitious agents that may be present at levels less than the limit of detection of analytical methods used to detect them, and is not intended to be a validated sterilization process.

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Introduction to ISIA

The International Serum Industry Association (ISIA) was founded in 2006 to represent collectors, producers, sellers, distributors, and end-users of animal serum and other animal-derived materials worldwide. Currently,

IMAGE: A digitally colorized transmission electron micrograph of the West Nile virus, a flavivirus. (Credit: Cynthia Goldsmith.)
<http://phil.cdc.gov/phil/details.asp?pid=10701>.

Workgroup Participants

In an attempt to fully represent all of the various interests of serum suppliers, irradiators, customers (end-users), expert scientists, and experts in regulatory matters, a dedicated group has been assembled to address this series of topics. The various participants and the industries they represent are detailed in **Table 1**.

Serum and its Role in Cell Culture

Serum and other blood-derived products play a critical role in the biomedical and biopharmaceutical arenas. These products are essential tools in many applications such as:

- Biomedical research
- Components in diagnostic kits
- Controls for drug discovery and drug compound development
- Understanding the pharmacokinetics and pharmacodynamics of newly discovered compounds and their effects on disease processes
- Cell culture-based safety testing of pharmaceuticals and cosmetics
- In growth media for cells in the production of human and veterinary vaccines and therapeutic proteins

Serum and other animal blood-derived products continue to be indispensable in modern medical biotechnology.

Among the most commonly used blood-derived products are:

- Whole blood
- Serum
- Plasma
- Immunoglobulins
- Albumin
- Transferrin
- Other Blood Fractions

Other animal by-products are used for the extraction of components such as enzymes and other naturally occurring proteins, which are also used in the biomedical arena. It should be remembered that animal blood is a by-product of the meat industry and, as such, is sourced from animals fit for human consumption

The worldwide biopharmaceutical industry is valued at more than 100 billion US dollars and is growing at greater than 10% per annum.^[2] Serum, plasma, and whole blood from a large variety of animal sources are used by the biopharmaceutical industry daily. Bovine-sourced products are the most commonly used products to supplement growth media. For the purposes of this document, we will concentrate on the use of animal serum, as this is one of the major animal by-products used in this application.

TABLE 1. Workgroup participants.

Participant	Affiliation	Representing
Sue Brown	TCS Biosciences	Supplier
Bart Croonenborghs	Sterigenics	Irradiator
James Dunster	Moregate BioTech	Supplier
Debbie Elms	Thermo Fisher Scientific	Supplier
Randy Fitzgerald	Proliant	Supplier
Greg Hansen	GE Healthcare	Supplier
Karl Hemmerich	Ageless Processing Technologies	End-user
Huw Hughes	Zoetis	End-user
Robert Klostermann	Merial	End-user
Raymond Nims	RMC Pharmaceutical Solutions	End-user
Mark Plavsic	Torque Therapeutics, Inc.	End-user
Andy Pratt	GE Healthcare	Irradiator
Mara Senescu	Steris	Irradiator
Rosemary Versteegen	ISIA	Industry
Martell Winters	Nelson Laboratories	End-user
Marc Wintgens	exelencia	Supplier

History of Serum Use

Serum has been used extensively as a growth medium supplement in cell culture for many years and its use for this purpose, particularly in the manufacture of vaccines, has greatly contributed to the battle against disease, both human and veterinary. From the proof of concept in the late 1880s^[3], cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. The growth of viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The Salk Polio Vaccine was one of the first products to be mass-produced using cell culture techniques in the early 1950s.^[4] Since then, serum has been used extensively by the global biopharmaceutical industry in the development and production of vaccines and biotherapeutics for both human and veterinary medicine. Mammalian cell culture processes are commonly used today for the production of recombinant proteins and glycoproteins, antibodies, and vaccines.

One of the most varied factors in culture systems is the growth medium, which provides the nutrients and/or the growth factors required. Serum is most often supplemented at a concentration of up to 10% in such culture media. The most commonly used bovine-derived serum are:^[5]

- **Fetal bovine serum (FBS)**—derived from fetal bovine blood collected at slaughterhouses. It has particular advantages: It typically has a low concentration of antibodies, which is important for *in vitro* antibody production, diagnostic applications, and vaccine manufacture. Also, fetal bovine serum has the ability to promote the growth, at low densities, of more fastidious cell lines.

- **Newborn calf serum (NBCS)**—a slaughterhouse-derived product obtained from calves less than 20 days old.

- **Calf serum (CS)**—also a slaughterhouse-derived product

collected from animals between 20 days and 12 months of age.

- **Adult bovine serum (ABS)**—another slaughterhouse-derived product obtained from animals between 12 and 36 months of age.
- **Donor bovine serum (DBS)**—a non-slaughterhouse-derived material collected from animals typically at 12–36 months of age that are bled under controlled conditions on a routine basis.

Serum derived from horses, pigs, chicken, sheep, goats, and numerous other species are also used in modern cell culture, predominantly in the manufacturing of veterinary vaccines.

Serum Risk and Risk Management

One of the most common routes for introducing adventitious agents into cell cultures is through animal-derived medium supplements and reagents. The possibility of the introduction and replication of adventitious agents during cell culture has long been recognized as a potential risk that must be managed accordingly. As a result, most regulatory bodies allow the use of animal serum and other animal-derived materials only when their use can be justified because there is no viable alternative.^[5,6] In recent years, advances in testing and filtration technology have helped in the management and mitigation of such risks.

Serum Testing for Adventitious Agents

Each serum lot must be tested for sterility, mycoplasma, and adventitious viruses. A lot (or batch) is made up of serum pooled from many animals, with traceability of blood to the collection dates and abattoir sources (or donor herds, depending on the collection strategy). Virus testing is typically performed in accordance with USA (9 Code of Federal Regulations)^[7], United States Pharmacopeia^[8], European Medicines Agency^[5,9], European Pharmacopoeia^[10], or World Health Organization^[6] testing requirements, and shown to be free of detectable agents (“not detected”) in the test. If serum is positive for an adventitious agent, for example, bovine viral diarrhoea virus (BVDV), and the batch is going to be irradiated, that irradiation process (dose) must be shown to be capable of inactivating BVDV at the quantity present in the batch.

Attempts to Replace Serum

As a result of the theoretical adventitious agent risk associated with use of serum in cell culture, many attempts have been made to remove or replace animal-derived materials in mammalian cell culture. In the late 1980s and early 1990s, the search for the holy grail of cell culture, the one culture medium that could replace medium supplemented with serum, cost thousands of research hours and millions of dollars, and proved to be only partially successful. Since that time, it has become apparent that animal-free media or serum replacements can be developed for certain cell

lines and applications, but not for others. It should also be recognized that animal material-free replacements are not without their own concerns. Plant-derived materials, for example, can introduce both animal- and plant-derived adventitious agents into cell cultures. Similarly, recombinant molecules used as media components can also have associated risks, depending upon the processes used to produce them.^[11]

Assuring Traceability

The fact that serum is still a necessary component in some biotechnology manufacturing processes has raised further questions in regard to reliability of geographic origin and possible adulteration. ISIA has implemented a traceability certification program designed to demonstrate traceability from abattoir to the end-user.^[5] This is based on an audit performed by an independent, approved third-party auditor according to an approved audit plan and using a detailed audit checklist. As of the time this article was written, 69% of those companies who have been ISIA members for over two years are either traceability certified or are in the process of becoming so.^[12]

Risk Assessment

Risk assessments for the use of animal serum and other animal-derived materials in biologics manufacturing are typically expected by regulatory authorities.^[5,6] Such assessments should address various aspects of the serum manufacturing chain leading from procurement of starting materials through use, and these may be summarized as:

- Sourcing of donor serum
- Manufacturing/filtration
- Packaging
- Comprehensive testing using highly sensitive analytical methods with known limits of detection, targeting the various adventitious agents of concern
- Post-manufacturing treatment(s)
- Storage and transportation
- End-use
- Traceability
- Documentation and recordkeeping

The concern, therefore, of all interested parties, is that all serum has been sourced, handled, and treated to meet regulatory and specific customer criteria. Thus, we will focus on post-manufacturing treatment with the intent of describing the tactics that may be used to mitigate those risks identified during the risk assessment exercise. In particular, we will provide greater detail on the gamma irradiation treatment strategy.

Post-Manufacturing Treatment Alternatives

The collection, transfer, filtration, and processing of serum is a complex and exacting task, which must comply with strict international regulations governing the

treatment of livestock, good farming practices, safe donor selection and control (in case of donor serum), methods of slaughter, veterinary oversight, collection, processing, and testing methods and procedures.

Following these best practices, serum is collected, transferred, and processed, as well as analyzed using sensitive test methods with known limits of detection for the adventitious agents of concern. The various treatment methods can be employed to further mitigate the risk of adventitious agents that might be present at levels which are less than the limit of detection of the analytical methods. It should be noted that “not detected” does not mean “not present” in the test material, but signifies that if present, such contaminants are below the assay’s limit of detection.

Various post-manufacturing treatment methods have been used, including:

- Filtration using 0.2 µm (and smaller) pore size filters
- Ultraviolet (UV) irradiation
- Heat treatment
 - The traditional “water bath method”
 - High temperature short time (HTST)
- Chemical treatment
- Ionizing radiation

Filtration

Triple 0.1 µm filtration has become the standard method of aseptic processing for various types of serum. While this methodology will remove bacteria and most mollicutes, it cannot render serum free of all viruses. Nanofiltration using viral retentive filters (20 nm nominal pore size) can be effective for removal of even small viruses such as parvoviruses. However, it suffers from scalability and flux decay with serum materials. Therefore, nanofiltration it is not suitable for large-scale processing of serum products.

UV Treatment

Ultraviolet irradiation (typically 254 nm) has been shown to be effective for pathogen reduction.^[13] The method is especially effective for larger microbes such as bacteria and mollicutes, but is also effective for most viruses. It is commonly employed for high-volume water disinfection. UV irradiation has also demonstrated efficacy in small-scale experiments aimed at clearing adventitious agents from cell culture media. However, it has not yet been optimized to effectively handle the larger volumes of media needed for commercial-scale bioproduction, and further technology development is needed.^[14]

HTST Treatment

Effective viral load reduction has been demonstrated for most viruses following HTST treatment. The most heat-resistant viruses, such as parvoviruses, require temperatures exceeding 115 °C.^[15,16] A significant issue with this modality, however, is the fact that the biological activity of the serum can be seriously compromised at

the relatively high temperatures required for treatment efficacy. This can potentially result in significantly reduced cell growth with various cell lines.

Traditional Heat Inactivation

Routine heat treatment of animal serum is performed in many cell culture labs as one of their normal procedures, and is included in many biomanufacturing protocols. A wide range of temperatures ranging from 45–62 °C, and times from 15–60 minutes may be called for. The most common methodology requires the heating of serum at 56 °C for 30 minutes.

Some well-designed and controlled studies^[17] have shown that growth characteristics for greater than 50% of cell lines tested are negatively impacted by serum heat inactivation. Variability has been identified in the: (1) exact temperatures and exposure times used; (2) mixing of the serum in the bottles; and (3) depth of water in the water bath relative to the height of the serum bottles. These factors, just to name a few, could all negatively impact the biological activity of the serum post-treatment. For these reasons, heat inactivation of FBS is not an ISIA-recommended practice unless it has been shown to be necessary for the specific cell culture application. It should be noted, however, that heat inactivation may be required to achieve complement inactivation in calf and adult bovine serum for use in some applications.

Chemical Treatment

Serum treatments with chemicals such as ethyleneimine or beta-propiolactone (BPL) have also been used as strategies to mitigate risk from adventitious agents in specific applications. Chemical treatment processes have specific hazards that must be managed to assure safety of the operators and the treated serum, and are not commonly used commercially on a large scale. Ozone technology (serum ozonation) is not suitable for serum treatment as it causes severe oxidation/peroxidation of serum components essential to cell culture growth, significantly reducing the serum’s performance.

Ionizing Radiation

The various forms of ionizing radiation that have the potential to reduce microbial and viral burden in serum include: electron beam, X-irradiation, and gamma irradiation.

- **Electron beam** utilizes high energy electrons to cause inactivation of contaminants. The electron beam does not, however, have the penetrating ability necessary to irradiate large finished bottles (*e.g.*, 500 mL and 1 L plastic bottles) of serum.
- **X-irradiation** theoretically should combine the best characteristics of electron beam and gamma irradiation. However, the commercial application of X-irradiation for this purpose has been limited because of the lack of

facilities that can handle large batches of serum.

- **Gamma irradiation** has both the penetrating power and ease of handling that allow it to be routinely used for pathogen reduction in finished serum bottles.

Gamma Irradiation

Gamma irradiation is the most commonly employed post-manufacturing approach for pathogen reduction in animal serum. The mechanisms of action (MOA) for pathogen reduction, and the efficacy of the approach for various pathogens, are discussed in a companion paper in this series.^[18] Guidance on dose-setting is also available.^[1] One reason why the gamma irradiation method has been commonly used for pathogen reduction in serum is that it may be performed on serum in the original product containers. Also, when done at extremely low temperatures, the performance capabilities of the serum are relatively unaffected. Cold chain management is essential during the steps leading up to and following irradiation so that the essential characteristics of serum will remain intact. This will also be a topic for a future article in this series.

Gamma Irradiation Technology and Irradiator Design

In the gamma irradiation process, serum products are exposed to a source of radiation. Gamma irradiators consist of four major components:

- (1) A biological shield
- (2) A source of radiation
- (3) A product handling system
- (4) A control and safety system

Biological Shield

The biological shield is the structure which contains the radiation source and provides attenuation of any radiation fields to levels which are safe for operators working outside the shield area. The shield is most often constructed of concrete, with an inner chamber containing the radiation source, and one or more intervening sections through which the product passes to enter the inner chamber. The shield may also be constructed of combinations of steel and/or lead, in addition to or as an alternative to concrete, as long as the resulting radiation fields outside the shield meet regulatory safety guidelines^[19,20] when the irradiator is operating. The interior area of the biological shield and the radiation source are often referred to as the "source pass." This is the working part of the irradiator where the irradiation exposure/processing actually takes place. Another term commonly utilized for this area is "the cell."

Radiation Source

The source of radiation in most gamma irradiators is cobalt-60 in the form of doubly encapsulated stainless steel source "pencils." Multiple pencils are held in a source

rack (frame) in defined and validated positions. The source rack is stored in a pool of water, attenuating the radiation effects when the irradiator is not in use, to ensure the safety of facility personnel. During irradiation, the source is lifted out of the pool and products are moved around it in an area of the source pass. The source is raised and lowered using a pneumatic hoist.

Product Handling (Processing Transport) System

The product handling system transports the products into the irradiator, to the source, and then back out again. Product is loaded into specially designed irradiation containers which may be tote boxes, hanging carriers, or pallets. The irradiation containers enter the shield through a maze-like section, which dissipates any possible exposure, moving into the inner chamber where they are indexed around the source, stopping at defined locations on both sides of the source, and then back outside of the shield where they are off-loaded and readied for release. The amount of radiation dose received by the product is a function of the design of the irradiator, the activity (intensity) of the radiation source, the density of the product (as loaded in its container), and the time spent in each position around the source.

Control and Safety System

The control system of an irradiator is designed to provide both operational and safety functions. Multiple redundant safeguards are in place to ensure that access to the irradiator is not allowed during operation, as well as operational health and safety controls around the product handling system. Modern irradiators are designed using a programmable logic controller platform for enhanced process control and monitoring. While rare, faults and events are captured in a database and can be viewed on a computer screen for normal operation monitoring and troubleshooting analysis.

Different Irradiator Designs

Gamma irradiators are designed such that the product being irradiated absorbs as much of the radiation from the source as possible, while at the same time providing an even distribution of absorbed dose within the product.

Tote System. The most cost-efficient irradiator designs, providing acceptable dose uniformity, are usually "tote systems" where product travels in many laps and many layers around the source to maximize the amount of radiation that is absorbed. While efficient, these irradiators can be difficult to operate because of the amount of product with similar density and dose requirements that must be in the source pass at all times.

Carrier System. These irradiators are designed with carriers to convey the product around the radiation source in a single level, usually making a single pass around the radiation source. While these systems are less cost-efficient, the "carrier system" design provides flexibility in processing

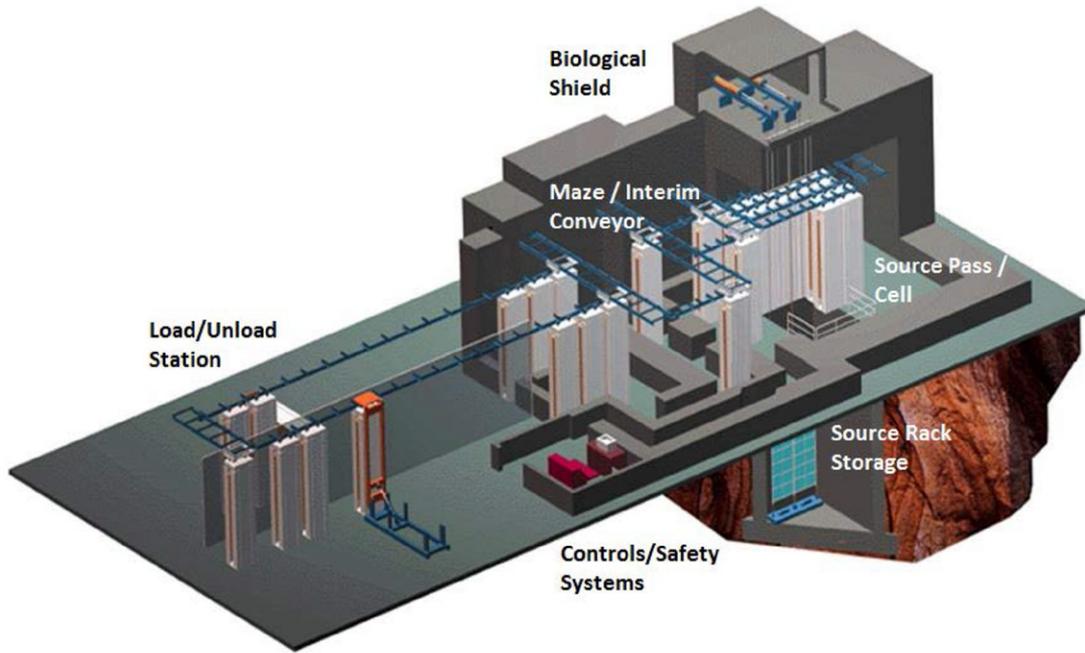


FIGURE 1. Schematic of a batch irradiator.

a variety of products with unique requirements.

Batch System. Even greater flexibility and dose uniformity in processing products of heterogeneous density can be achieved by utilizing a “carrier batch” irradiator design. In a batch irradiator, all product is loaded into the cell and irradiated under equal conditions. By utilizing product loading patterns to reduce product “effective” density while reducing the stack height of the product, very tight dose uniformity ratios (DURs) can be achieved (Figure 1).

Pallet Systems. These irradiator systems are not well-suited to serum processing due to their higher product “effective” density, and thus resultant larger product DUR.

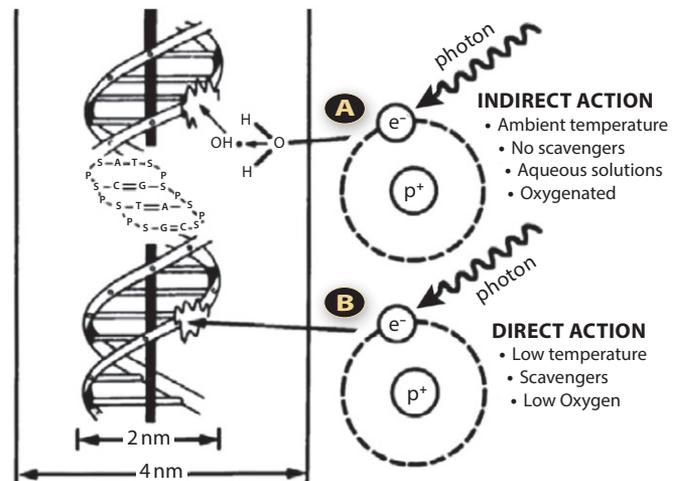
Off-Carrier Systems. The descriptions above have dealt primarily with commercial-grade irradiators. It should be noted that off-carrier irradiators (research loop irradiators, as described in the AAMI documents⁽¹¹⁾) are useful for special projects requiring narrow dose ranges (such as viral clearance studies) and small numbers of test samples or product.

Mechanisms of Action for Gamma Irradiation

It is well-known that gamma irradiation can lead to alterations in the materials being treated. These, and the desired pathogen reduction effects, are the result of the radiation’s effects in the solutions which include both indirect (Figure 2A) and direct actions (Figure 2B). The direct action consists of interactions of the radiation with polymeric substrates such as the nucleic acids of the microorganisms. This can take the form of base mutations, strand cross-linking, and strand breakage. The indirect action is a result of interactions of the radiation with the intracellular or extracellular milieu, and includes the creation of short-lived free-radicals (e.g., hydroxyl radicals, hydrogen

atoms, and solvated electrons) derived from the radiolysis of water. The inactivation of microorganisms through this pathway is dependent upon the ability of the free-radical products to diffuse to macromolecular targets, and is subject to scavenging by solutes such as proteins.

The extent of microorganism inactivation by gamma irradiation in aqueous solution can therefore be a function: (a) of not only the microorganism itself (especially its radiation target size) and the strandedness (single vs. double) of its genome; but also (b) of the nature and composition of the solute and the oxygen content and temperature of the solution. Scavengers of free-radicals produced during irradiation can reduce the indirect inactivating action (i.e., that which was mediated by free-radicals)



FIGURES 2A and B. Schematic of the inactivating mechanisms of gamma irradiation.

of a given dose of gamma irradiation. In contrast, in the presence of oxygen and at higher temperatures, the indirect inactivating action is enhanced. In the presence of high scavenger levels and freezing temperatures, the indirect effects should be minimized and the direct effects should therefore predominate. Under the latter conditions, the inactivation of microorganisms such as viruses and mollicutes by gamma irradiation should be first-order with respect to dose, resulting in linear radiation dose vs. inactivation curves. A minimization of the indirect effects of gamma irradiation through, for instance, irradiation at low temperatures, would also be expected to protect the protein and other radiation-sensitive components of irradiated animal serum. For these reasons, gamma irradiation of serum products should be performed on deeply frozen serum at temperatures as close to -70°C as practical.^[21]

Conclusions

The processes involved for the gamma irradiation of serum are not extremely transparent to serum end-users or to other parties in the cell culture and regulatory communities.

- Who actually does the irradiation?
- How is the dose selected and controlled?
- Who validates the process and how is this done?
- How effective is the irradiation for achieving reduction of bacteria, mycoplasma, and viruses in serum — and how is this determined?
- Who regulates the irradiation process?
- What are the potential impacts on serum performance and how can these be determined?

These are the questions that we hope to clarify in this series of articles to appear in BioProcessing Journal. This introduction to gamma irradiation is the first paper of the series.

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About the Authors

Rosemary J. Versteegen, PhD*, is Chief Executive Officer, International Serum Industry Association, McHenry, Maryland USA.

Mark Plavsic, PhD, DVM, is Head of Product Development and Manufacturing, Torque Therapeutics, Inc., Cambridge, Massachusetts USA.

Raymond Nims, PhD, is Senior Consultant, RMC Pharmaceutical Solutions, Inc., Longmont, Colorado USA.

Robert Klostermann, BS, is a Senior Manager in the Corporate Quality Assurance Third Party Management group, Merial, Athens, Georgia USA.

Karl Hemmerich is President of Ageless Processing Technologies, Sandy, Utah USA.

*Corresponding Author:

Email: rjv@serumindustry.org | Phone: 301-387-4967

Website: www.serumindustry.org

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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