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Research Report

Validated Gamma Radiated Serum Products

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Abstract

SAFC Biosciences has established process parameters and controls for maximum inactivation of microbial contaminants while maintaining product integrity through its validation of the gamma irradiation process for serum products. The validation study evaluated package configurations, process temperatures, radiation dose response and radiation distribution within the packages. The study was conducted at both pilot scale (100 mL samples) and production scale (500 mL, 1000 mL, 3 L and 4 L samples).

Serum samples were spiked with bacteria, fungi, mycoplasma, bacteriophage and viral contaminants and exposed to varying doses of radiation to determine the dose inactivation response. Fifteen (15) kilograys (kGy) inactivated greater than 5 logs of bacteria, fungi and mycoplasma. The viruses were significantly reduced at a radiation dose range between 25 - 35 kGy. Product performance was evaluated utilizing 3 separate lots of fetal bovine serum (FBS) exposed to 25 - 35 kGy radiation. Maintenance of product integrity was determined by utilizing standard cell culture assay techniques, i.e., multiple passage, blue tetrazolium salt cell proliferation assay (MTT), plating efficiency and cloning efficiency. The biochemical profiles were also compared to non-irradiated controls. Cell growth assays clearly demonstrate that when properly radiated, animal serum can maintain its ability to promote cell growth.

Introduction

Viral clearance is a major concern for manufacturers of both human and animal biological products. The first step in viral clearance is to reduce the potential risks associated with the animal-derived raw materials used in the manufacturing process. Regulatory bodies are now requiring up to 15 log reductions in viral load. In order to achieve this level of safety assurance, the process stream must be extensive and robust and raw material must be 'clean.' To ensure that the raw material carries a low level of risk, the material must first be tested for absence of microbial contaminants and can also be treated for increased reduction of potential undetectable organisms.

Gamma irradiation is an accepted treatment method for animal-derived material that has been demonstrated to remove up to 6 logs of extraneous agents. The process must be tightly controlled in order to be consistent, reproducible, effective and efficient, while maintaining product performance. To establish these process controls, SAFC Biosciences conducted a validation study that determined the optimal temperature, established a standard packaging configuration, set specific time limits, determined the minimum and maximum radiation exposure within the product itself and established a radiation dose range that protects product integrity while maximizing inactivation of microbial contaminants.

The validation study was conducted at pilot scale and confirmed at the production scale levels. This study demonstrated FBS radiated at 25 - 35 kGy under tight process controls will perform equivalently to non-radiated controls and will inactivate 5 - 6 logs of the common cattle viruses: *Bovine Viral Diarrhea* (BVD), *Infectious Bovine Rhinotracheitis* (IBR) and *Parainfluenza Type 3* (PI_3). Therefore, biological manufacturers can use SAFC Biosciences' serum products with a high level of confidence that extraneous agents are not being introduced into their products through this animal-derived raw material.

Materials and Methods

FBS, Catalog. No. 12103.
Donor horse serum (DHS), prescreened for neutralizing antibodies, Catalog No. 12449.
Saline (water containing 0.85% NaCl).

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Cell lines: 3T3 (ATCC CCL92), A549 (ATCC CCL185), CHO (ATCC CCL61), L243 (ATCC HB55), MDBK (ATCC CCL 22), Sp2/0 (ATCC CRL1581) and Vero (ATCC CCL81)

Study Organisms

Organism	Type	Size
Escherichia coli (E.coli)	Gram negative rod	1.5 - 4.0 µm
Bacillus pumilus (B.pumilus)	Gram positive rod Spore former	0.6 - 2.5 µm
Candida albicans (C.albicans)	Fungus (dimorphic)	2 - 3 µm
Acholeplasma laidlawii (A.laidlawii)	Mycoplasma	0.3 - 0.8 µm
JH Strauss	Bacteriophage	24 - 26 nm
Bovine Viral Diarrhea (BVD)	Togavirus (cytopathic strain) ssRNA	30 - 60 nm
Parainfluenza Type 3 (PI_3)	Paramyxovirus ssRNA	100 - 250 nm
Infectious Bovine Rhinotracheitis (IBR)	Herpes dsDNA	100 nm
Porcine Parvovirus (PPV)	Parvovirus ssDNA non-enveloped	18 - 24 nm
Minute Virus of Mice (MVM)	Parvovirus ssDNA	25 nm
Bluetongue Virus (BTV)	Orbivirus dsRNA	60 - 80 nm
Feline Leukemia Virus (FeLV)	Retrovirus RNA	100 - 120 nm

Rationale for Microorganism Selection

The organisms selected are naturally occurring potential contaminants from either the animals themselves, i.e., *Bovine Viral Diarrhea*, or from the environment during the collection process, i.e., *Minute Virus of Mice*. In order to represent a wide spectrum of virus types, both enveloped and non-enveloped viruses, RNA and DNA viruses and relatively large and small viruses were included in the study. Some organisms, such as *Bovine Leukemia Virus*, are of interest but are difficult to culture *in vitro*. By choosing model viruses of the same type, but from different species, information about the inactivation properties of these adventitious agents can be accurately assessed. In these studies *Feline Leukemia Virus* was used as a model for *Bovine Leukemia Virus* and *Porcine Parvovirus* was used in place of *Bovine Parvovirus*.

Temperature Optimization

FBS samples were irradiated at 4 separate temperature ranges. The irradiated samples and non-radiated control were then evaluated for growth promotion, plating efficiency and protein production, as well as physical and biochemical characteristics.

Coolant Validation

Saline solutions were sent to the irradiation facility with varying amounts of coolant. Thermocouples were used to monitor the temperatures at various times throughout the process. This information was used to determine the amount of coolant necessary to maintain the optimum product temperature.

Pilot Scale Inactivation

100 mL DHS samples, prescreened for neutralizing antibodies, were spiked with known quantities of *E. coli, B. pumilus, C. albicans, A. laidlawii,* JH Strauss, BVD, PI_3, IBR, PPV and MVM. These samples were then irradiated at a wide range of dosage levels. Radiated samples and non-radiated controls were then screened for the presence of residual titers.

Production Scale Inactivation

500 mL, 1000 mL, 3 L and 4 L samples of DHS were spiked with either BVD, PI_3, IBR, or MVM. These samples were then radiated at 25 - 35 kGy and assayed for residual titers. 500 mL samples spiked with BTV and FeLV were also radiated and assayed in a continuation of the validation. Samples in which no viral titer was detected were serially passaged on susceptible cell cultures to give a sampling confidence of > 95% based on a theoretical viral load of 10 particles per liter.

Product Integrity Testing

Three lots of FBS in 500 mL, 1000 mL, 3 L and 4 L containers were irradiated at 25 - 35 kGy. All lots were then evaluated for cell growth by performing multiple passage assays, MTT assays (actively growing cells produce a dye that is spectrophotometrically quantified), plating efficiency and cloning efficiency. All testing was done in parallel with matching non-irradiated controls.

Process Controls

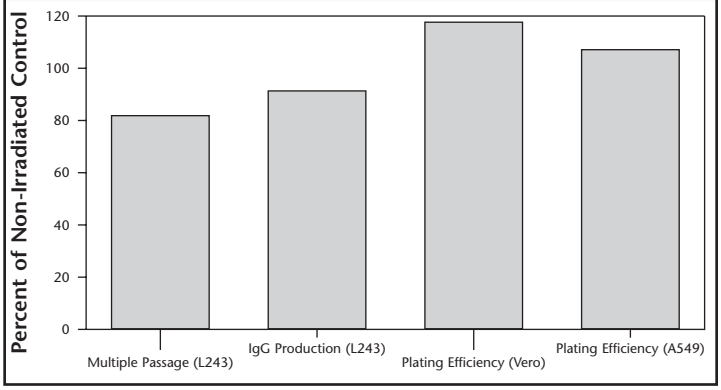
Using a proprietary dose mapping model, the actual radiation dose delivered to the product was monitored and controlled. In addition, qualified SAFC Biosciences' personnel packed each container according to a standardized configuration before shipment on an SAFC Biosciences' truck to the irradiation facility.

Results

Temperature Optimization

L243 hybridomas were used to evaluate multiple passage growth and the production of IgG using serum radiated at various temperatures. Plating efficiency data was obtained using Vero and A549 cultures. As Table 1 shows, all results from serum radiated at the optimum temperature were ≥ 82% of the non-radiated control.

Table 1: Performance of Serum Radiated at Optimum Temperature



Pilot Scale Inactivation

Spiked serum samples were exposed to a 0, 7, 15, 25, 35, or 45 kGy ± 1.3 kGy dose of radiation. The log reductions of each organism experienced at these dosage levels appear in Table 2.

Table 2: Pilot Scale Inactivation Results

	Dosage (kGy)					
Organism	**0[a]**	**7**	**15**	**25**	**35**	**45**
E. coli	> 6.0	> 6.13	> 6.13	> 6.13	> 6.13	> 6.13
B. pumilus	> 6.0	> 6.15	> 6.15	> 6.15	> 6.15	> 6.15
C. albicans	> 6.0	3.84	> 6.27	> 6.27	> 6.27	> 6.27
A. laidlawii	> 8.0	6.00	> 8.0	> 8.0	> 8.0	> 8.0
JH Strauss	8.5	0.70	1.02	0.99	2.24	2.11
BVD	6.0	1.40	2.81	5.84	5.84	5.84
IBR	6.0	2.31	4.59	5.31	5.31	5.31
PI_3	6.0	1.16	3.19	5.57	5.57-6.07	6.07
MVM	5.0	0.53	0.99	1.62	2.59	3.24
PPV	5.0	0.50	0.69	1.19	1.69	2.69

[a]Starting titer (log_{10})

Production Scale Inactivation

Table 3 shows the amount of inactivation achieved after spiked serum samples were radiated at 25 - 35 kGy and assayed for residual titers.

Table 3: Production Scale Inactivation Results

Organism	0 kGy	25 - 35 kGy	Log Reduction
BVD	4.32[a]	None detected	≥ 4.32
IBR	4.69	None detected	≥ 4.69
PI^3	7.13	None detected	≥ 7.13
MVM	4.60	0.80	3.80
BTV	6.42	3.11	3.31
FeLV	5.87	2.58	3.29

[a]Starting titer (log_{10})

Product Integrity Testing

Three cell lines (3T3, MDBK and L243) were passaged 3 times in irradiated serum and non-irradiated controls. The results for 1 of the 3 lots are summarized in Table 4. Irradiated and non-irradiated sera showed no significant difference in growth promotion capabilities. Similar results were obtained with the other 2 lots of serum.

Table 4: Multiple Passage Growth Results

Cell Line	Serum Sample	Average Cell Count (x 10^6 cells/flask)	Percent of Control
3T3 (Fibroblast)	Lot X Control	20.69	–
	Lot X Irradiated	21.84	106
MDBK (Epithelial)	Lot X Control	46.90	–
	Lot X Irradiated	53.25	114
L243 (Hybridoma)	Lot X Control	10.43	–
	Lot X Irradiated	10.69	102

Results of the MTT growth/cytotoxicity, cloning efficiency and plating efficiency studies also demonstrated no major differences in the performance of radiated and non-radiated serum. All samples performed at ≥ 83% of the corresponding control serum, with the vast majority performing at levels ≥ 99% of the control.

Conclusions

Exposure to gamma radiation at 25 - 35 kGy under tightly controlled conditions significantly reduces the levels of many types of microorganisms, including bacteria, fungi, mycoplasma, bacteriophage and viruses. Serum performance is minimally affected by the gamma irradiation process. No significant differences in growth promotion, cloning and plating efficiencies, or IgG production were observed in comparison to non-radiated controls.

In conjunction with extensive prescreening of raw serum which includes full 9CFR 113.53 testing, gamma radiated serum increases the level of assurance or "safety" when using an animal-derived product. While no inactivation process is 100% effective, gamma radiation conserves product performance and is consistent, reproducible, controllable, minimally intrusive and efficient in reducing levels of contaminating microorganisms.

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