Temperature Controlled Gamma Irradiation

PRODUCT NEWS

Gamma Irradiation

To minimise the risk of viruses in animal-origin material, gamma irradiation is the preferred choice of the regulators. Gamma irradiated Gibco® Foetal Bovine Serum (FBS) meets the requirements of the European Medicines Agency (EMEA) guidelines for the use of bovine origin products in the manufacture of biopharmaceuticals. The CVMP guideline† recommends a minimum dose of 30kGy. However, different applications may require different dosage regimes and we have a range of validated protocols available (Table 1). We can customise our offering to meet your needs for pack size and dose range. Our validated process for gamma irradiation has been shown to inactivate common bovine viruses and mycoplasmas which may be present in sera. We have also demonstrated that when our temperature controlled method is used the physiochemical properties and the cell culture performance of serum is not significantly altered by gamma irradiation of up to 45kGy.

Table 1 - Table of Gamma Irradiation Options

Bottle Size	Dose Range (kGy)	Gamma Irradiation Method
100ml	25-36	Temperature Controlled
	25-42	Standard
	30-45	Temperature Controlled
	30-50	Standard
500ml	25-36	Temperature Controlled
	25-42	Standard
	35-50	Custom
	30-40	Temperature Controlled
	30-50	Custom
1000ml	25-36	Temperature Controlled
	25-42	Standard
	35-50	Custom
	30-45	Temperature Controlled
	30-50	Custom
4-5L	35-60	Custom
	35-60	Custom
	25-42	Custom
	25-42	Custom
	30-45	Custom
	35-55	Custom
	30-50	Custom
10L	25-42	Custom

Benefits of Gibco® Gamma Irradiated Serum:

- Validated inactivation of mycoplasmas and many viruses, including BVD, IBR, PI3, PPV and REO3
- Reduces contamination risk
- High levels of biological performance maintained
- Many dose ranges available for different pack sizes
- Irradiation performed using validated protocols
- Compliance with regulatory guidelines
- Scaleable

† EMEA/CVMP/743/00 Final Guideline on requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products.

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Virus Inactivation: Validation Study

The validation study was designed to measure the effectiveness of gamma-irradiation in reducing a viral burden many times greater than that expected to occur in natural serum samples.

A panel of five viruses with a variety of physico-chemical properties were selected for use in the study (Table 2).

Table 2 - Panel of Viruses for Virus Inactivation Study

Virus	Family	Properties	Name	Size (nm)
BVDV	Flaviviridae	ss-RNA, enveloped	Bovine Viral Diarrhoea Virus	30-60
IBR	Herpetoviridae	ds-DNA, enveloped	Infectious Bovine Rhinotracheitis	100
PI3	Paramyxoviridae	ss-RNA, enveloped	ParaInfluenza Virus 3	150
PPV	Parvoviridae	ss-DNA, non-enveloped	Porcine Parvovirus	18-26
REO3	Reoviridae	ds-RNA, non-enveloped	Reovirus 3	60-80

The panel chosen were representative examples of viruses which were most likely to be found in cattle, or where these were not practical, model viruses like Bovine Parvovirus and Reovirus 3 were selected. The inclusion of parvovirus and reovirus is particularly valuable as both are small viruses which are known to be very difficult to inactivate by both chemical and physical methods. The panel is also representative of those recommended by the regulatory authorities (Table 3).

Table 3 - Viruses for testing by the EMEA

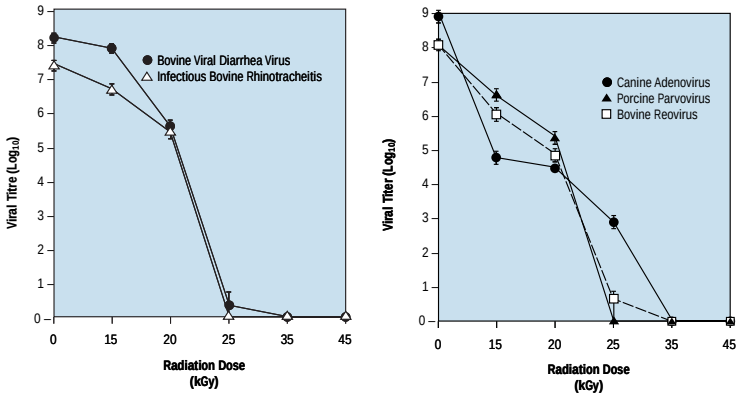
CPMP/BWP/1793/02 'Note for Guidance on the use of bovine serum in the manufacture of human biological medicinal products.'	CVMP/743/00-Final 'Guideline on requirements and controls applied to bovine serum used in the production of immunological veterinary medicinal products.'
Part 5.3.2 suggests testing for:	**Part 4.3.1 suggests:**
Bovine Adenovirus	Bovine Adenovirus
Bovine Parvovirus	Bovine Parvovirus
Bovine Respiratory Syncytial Virus	Bovine Respiratory Syncytial Virus*
Bovine Viral Diarrhoea Virus	Bovine Viral Diarrhoea Virus
Rabies Virus	Parainfluenza Virus 3
Reovirus 3	Reovirus 3
Bluetongue and related orbiviruses	IBR
	and those responsible for diseases exotic to Europe (e.g. Bluetongue)
	* From Part 4.3.2 'These tests should be carried out, a first time, before the inactivation treatment to assess the infectious titre of BVDv potentially present and ensure it is below the level that has been shown to be effectively inactivated in the validation tests. Secondary tests should be performed after the inactivation treatment at which time no virus should be detected in the final serum batch. These tests could be omitted if no virus is detected before inactivation treatment.'

Individual bottles of FBS were separately spiked with the viruses and then exposed to gamma-irradiation. Following treatment, the spiked samples were assayed for residual infectious virus, using standard quantitative cell culture assays.

Dose-Dependent Decline in Viral Titre

A dose-dependent decline in survival was seen for enveloped and non-enveloped viruses (figure 1). The viruses showed a significant decline in titre at 25kGy and were below the level of detection (0.5 TCID50/ml) at 35kGy. Reduction factors were 6.7- to 7-fold.

Figure 1 - Dose-dependent virus inactivation by gamma irradiation.



Enveloped or non-enveloped viruses were spiked into FBS and gamma-irradiated. Titres were determined by the quantitative plaque assay for enveloped and the $TCID_{50}$ assay for non-enveloped viruses. Results are mean ± SE for 3 lots of FBS.

Cell Growth at Single-Cell and Low-Cell Density

The growth-promoting activity of FBS is most rigorously tested when cells are plated at clonal densities (1 or 5 cells/well). Gamma irradiation did not decrease the ability of the FBS to support cell growth in these most rigorous conditions (Table 4). Also, gamma irradiated serum supported the growth of adherent cells seeded at low density (100 or 200 cells/well) at levels comparable to the non-irradiated sera (Table 4).

Table 4 - Cell growth at single-cell (cloning efficiency) or low-cell (plating efficiency) seeding densities.

Gamma-irradiation Dose (kGy)	Relative Cloning Efficiency SP2/0 Cells		Relative Plating Efficiency A549 Cells	
	10% FBS 1 cell/well	4% FBS 5 cells/well	10% FBS 100 cells/well	4% FBS 200 cells/well
0	1.12 ± 0.01	1.06 ± 0.01	0.89 ± 0.01	0.92 ± 0.01
25	1.07 ± 0.03	1.04 ± 0.02	0.97 ± 0.04	0.96 ± 0.03
35	1.05 ± 0.04	1.07 ± 0.02	0.92 ± 0.02	0.93 ± 0.02
45	1.11 ± 0.05	1.08 ± 0.01	0.94 ± 0.06	0.93 ± 0.03

Results are mean ± SE of 3 different lots of FBS.

Cell Growth in Multiple Passages

A panel of continuous cell lines routinely used in the production of animal vaccines was tested to determine if gamma-irradiated serum supported sequential subculturing. Growth was determined from the average of the second and third sequential subcultures of irradiated FBS compared to non-irradiated FBS to minimise any effect(s) due to serum carry-over from earlier maintenance of the cells. Cell morphology in the gamma irradiated FBS was consistent with that of the control cultures. VERO, WI-38 and MRC-5 cells grew equally well in irradiated or control FBS (Table 5). Of interest is that WI-38 and MRC-5 did not display any decline in growth as a function of gamma irradiated FBS, since these fibroblasts are used because of their sensitivities to toxic factors.

Table 5 - Long-term cell growth.

Cell Line	Radiation Dose (kGy)	Growth (Percent of Control)
VERO	15	97 ± 4
	20	102 ± 6
	25	90 ± 10
	35	91 ± 8
	45	86 ± 8*
WI-38	15	107 ± 6
	20	108 ± 3
	25	104 ± 6
	35	104 ± 6
	45	103 ± 11
MRC-5	15	99 ± 4
	20	104 ± 2
	25	99 ± 9
	35	96 ± 9
	45	98 ± 14

*$p \leq 0.05$ relative to control non-irradiated FBS.

Results are mean ± SE for passages 2 and 3 for 3 lots of FBS.

Temperature Control

Results show that unless gamma-irradiation is performed under temperature controlled conditions, the biological performance of FBS is impaired.

Gibco's irradiation process is optimised and is carried out according to a strictly controlled standard operating procedure, with both temperature and dose carefully monitored. Our dose mapping study has validated the delivery of a particular minimum dose within particular packaging configurations.

Serial passage and plating assays using BHK cells were performed on 5 different batches of FBS before and after irradiation. Two main conclusions can be drawn from the figures below.

1. when irradiated under non controlled conditions, the growth performance of the serum (S3) is consistently low.
2. when conditions are controlled throughout the irradiation process, the biological performance of the serum (S2) is comparable to the non-treated serum (S1).

Figure 2 - Effect of gamma-irradiation on BHK Planting

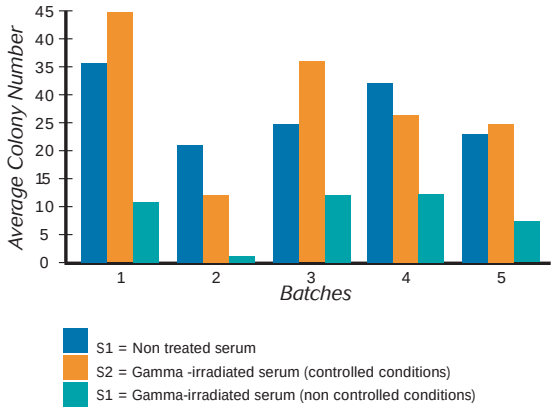
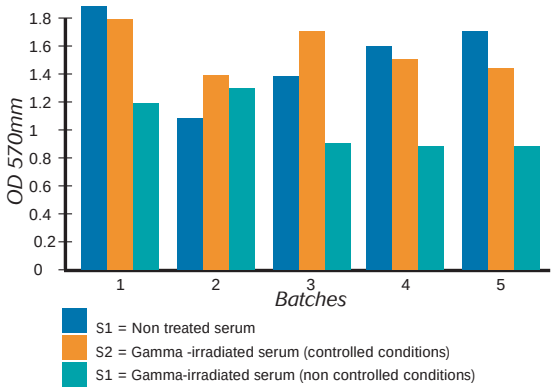


Figure 3 - Effect of gamma-irradiation on Serial Passage of BHK (passage no 3)



In all five batches tested, irradiation under non-temperature controlled conditions is detrimental to the efficacy of FBS (S3). This drop is not observed in the FBS irradiated under temperature controlled conditions (S2), with all batches showing growth results comparable to the non irradiated control batches (S1). This demonstrates that, in most of these applications, FBS performance for supporting the growth of cells in culture is not impaired, as long as it is irradiated in strictly controlled conditions.

Conclusion

These results demonstrate that gamma-irradiation can effectively be used in the inactivation of viral contamination with little loss of cell growth characteristics. Gibco® can provide documented evidence that its process can eliminate viruses with minimal loss of cell growth characteristics.

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