Gamma Irradiation and Heat Inactivation Comparison

Rosie Versteegen
Nicole Green

NOTHING WORKS LIKE SERUM

INTERNATIONAL SERUM INDUSTRY ASSOCIATION

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.
- This possible 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
- In recent years, advances in testing and filtration technology have helped in the management and mitigation of such risks.

Post-Manufacturing Treatments

- Filtration using 0.2 μm (and smaller) pore size filters
  - Not effective for viruses
- Ultraviolet (UV) irradiation
  - Not currently commercially available
- Heat inactivation/treatment
  - More later
- Chemical treatment
  - Not currently commercially available
- Ionizing radiation
  - Electron beam
    - Not enough penetrating capability for bottles
- X-irradiation
  - Not commercially available
- Gamma Irradiation
  - Has the penetrating power and ease of handling for routine use in viral load reduction for finished serum

Serum Testing for Adventitious Agents

- Each serum lot must be tested for all types of adventitious agents
  - Bacteria
  - Mycoplasma
  - Adventitious viruses.
- Bacteria are removed by filtration
- Most mycoplasma are also removed by filtration.
  - Gamma irradiation is highly effective
- Virus testing is typically performed in accordance with USDA 9 CFR, USP, EMAA Agency, EP or WHO requirements, and serum should be free of detectable agents.
- If serum is positive for an adventitious agent, then gamma irradiation is the method of choice for viral load reduction

Risk Management for Animal Serum

- If serum is positive for an adventitious agent, then gamma irradiation has been the method of choice for viral load reduction

- But what about heat inactivation?

Heat Inactivation

- Heat treatment of animal serum is a longstanding and normal procedure in many cell culture labs and is included in many biomanufacturing protocols.
- The most common methodology requires the heating of serum at 56°C for 30 minutes
  - Serum must be thawed in the refrigerator or on the bench and mixed well, as it stratifies on freezing
  - Capped and sealed bottles must be placed in a water bath deep enough to submerge all the serum.
  - Once the temperature of the bottles has reached 56 °C, they must be left in the water bath for 30 minutes and gently agitated periodically
- Heat inactivation can be performed in bulk as a custom order from some suppliers

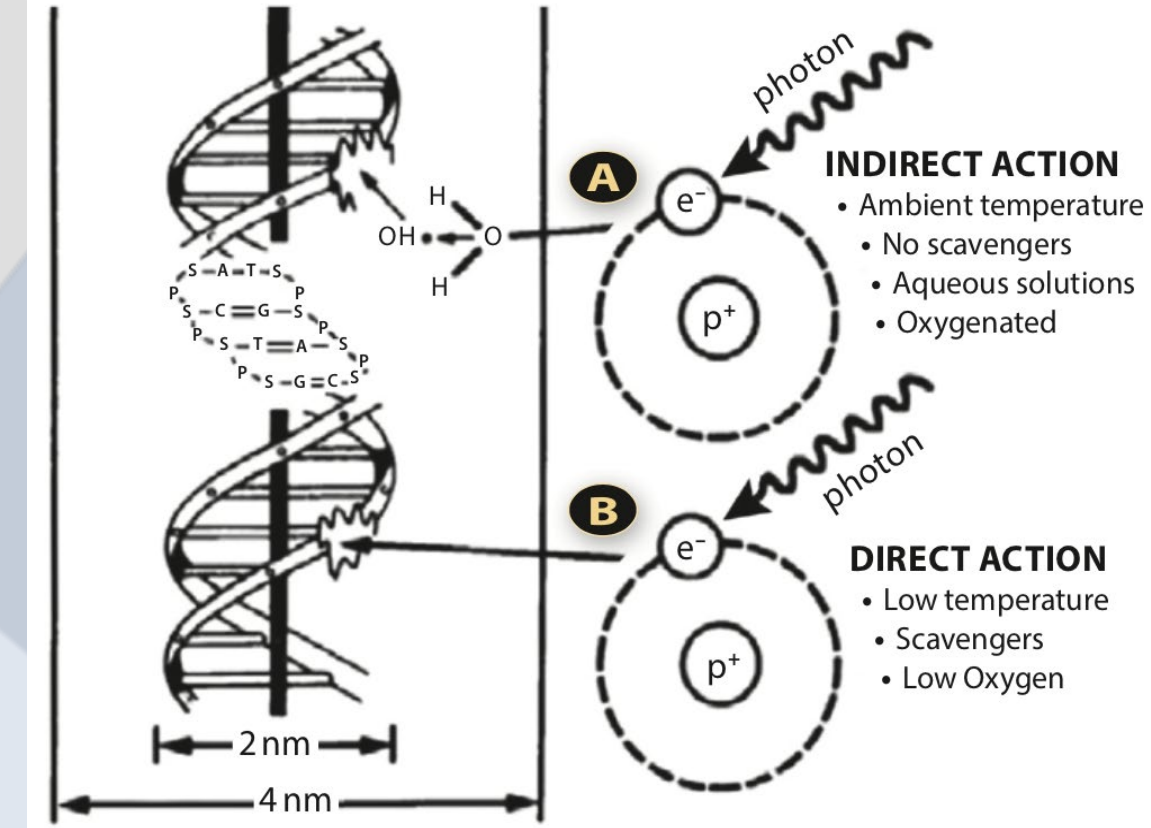
Heat Inactivation Concerns

- Well-designed and controlled studies on heat inactivation have shown that growth characteristics for greater than 50% of cell lines tested are negatively impacted by serum heat inactivation.
  - The variability inherent in the process includes:
    - Exact temperatures and exposure times used
    - Mixing of the serum in the bottles
    - Depth of water in the water bath relative to the height of the serum bottles
- This variability is compounded by the fact that temperatures ranging from 45–62°C, and times from 15–60 minutes may be required.

So what about Gamma Irradiation?

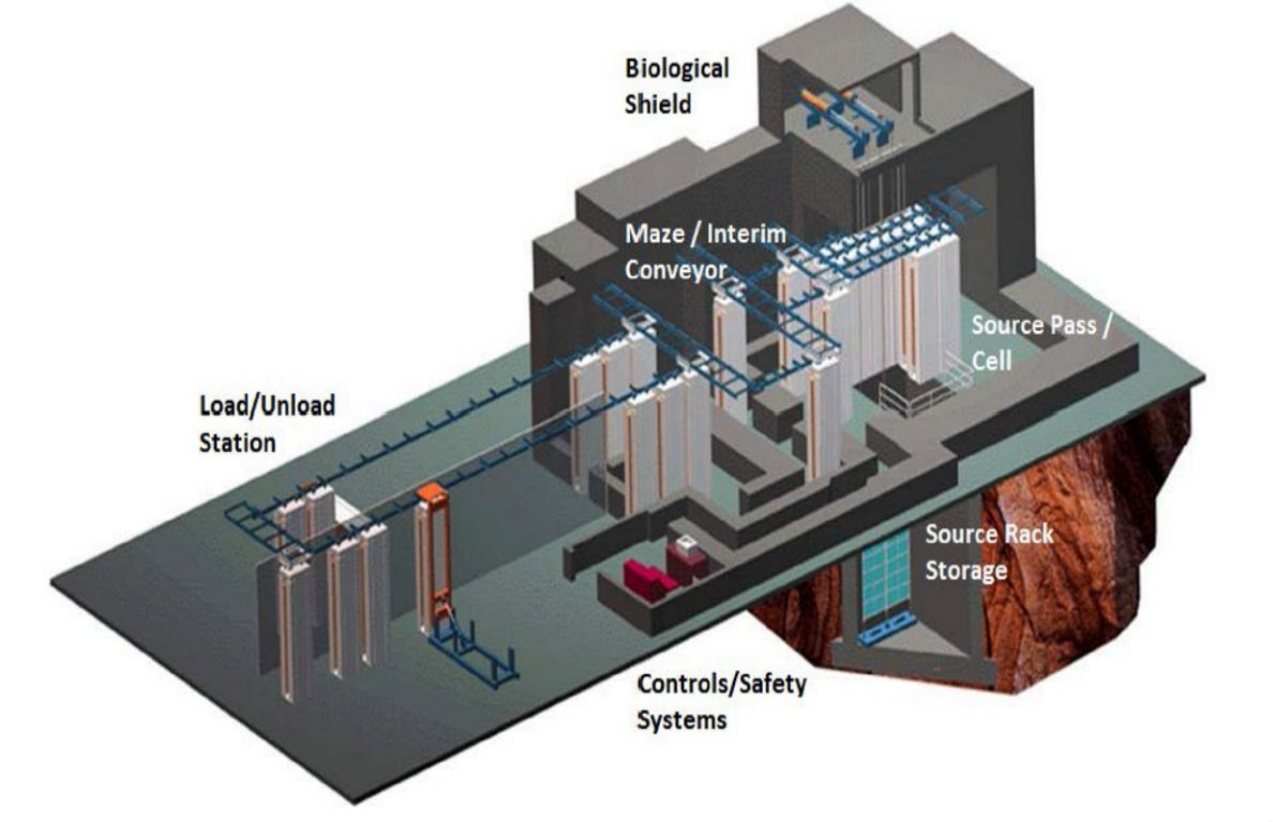
A more complex but better controlled methodology

Method of Action of Gamma Irradiation



- The direct mechanism minimizes unintended damage to critical animal serum components
- Irradiation in sealed product containers at low temperature (typically – 60 °C or lower) results in inactivation of micro-organisms in a manner that is first-order with respect to radiation dose.
- Gamma irradiation is effective on all but very small non-enveloped viruses

Irradiation Process

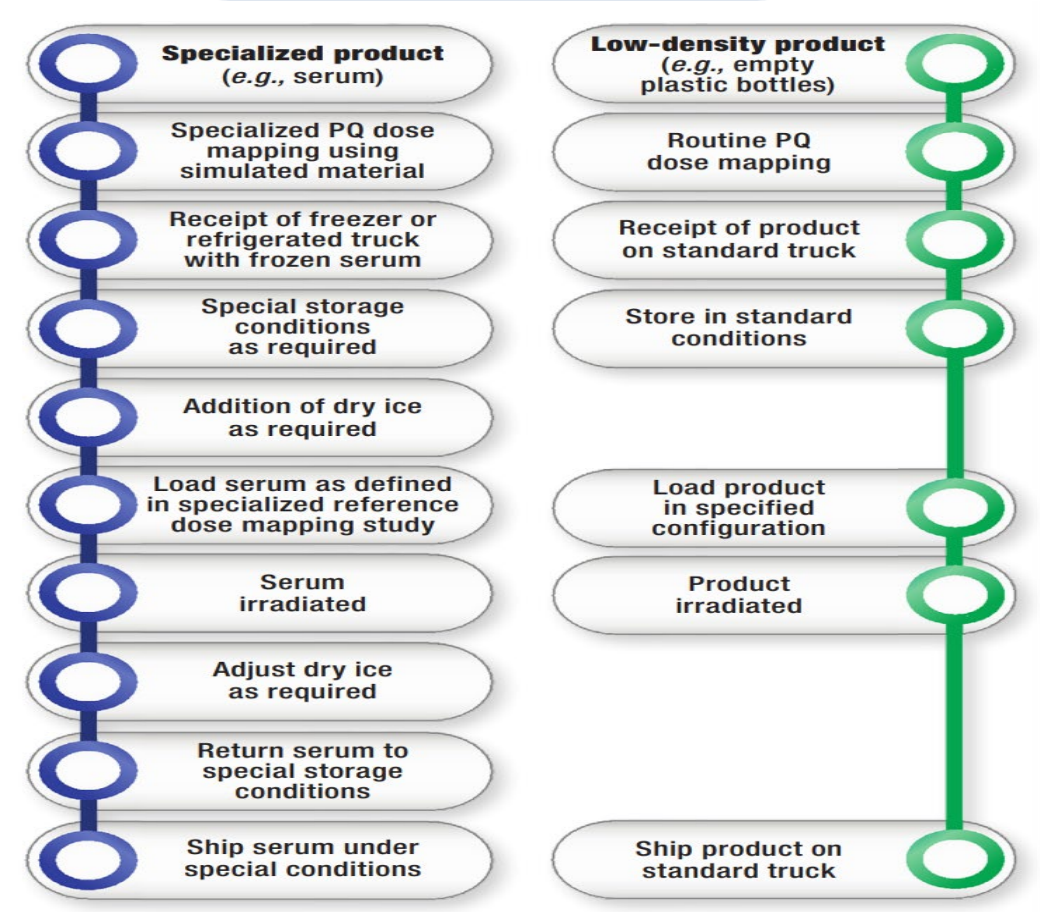


- Serum is loaded into specially designed and configured irradiation containers
- Dry ice is added to reach very cold temperatures
- Containers enter the shield through a maze-like section
- Move into the inner chamber
- Index around the source, stopping at defined locations on both sides of the source
- Move back outside of the shield and off-loaded
- Certificate of Irradiation generated

The radiation dose received 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)
- the time spent in each position around the source

Irradiation of Specialty Products



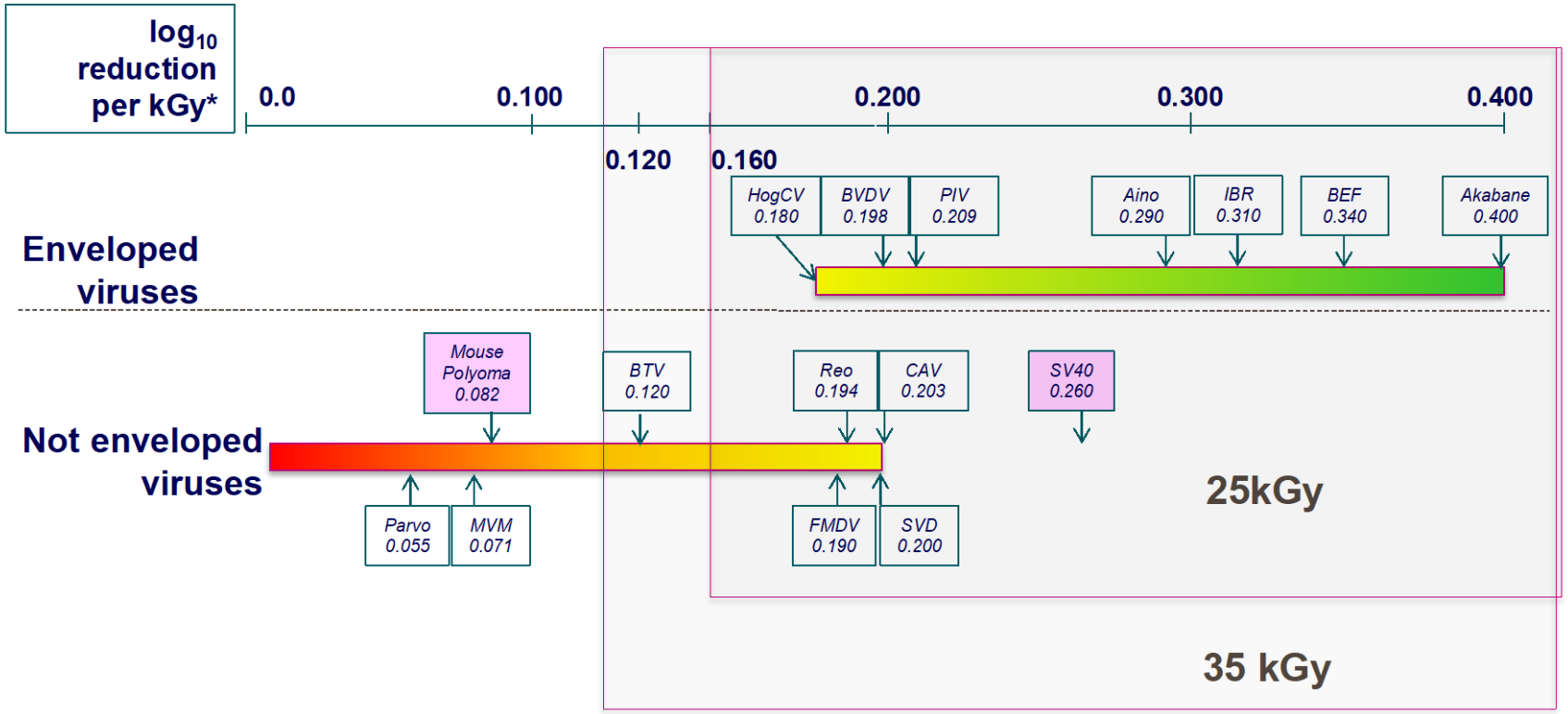
- Significantly more complicated
- Dry ice needs to be reloaded
- Cannot be run unattended
- Leads to difficulty in scheduling

Irradiation Facts

- A reduction of 4 logs is considered effective based on the viral load generally found in serum
- Small unenveloped viruses are the least sensitive to gamma irradiation
  - Higher doses will destroy the biological activity of the serum
  - Alternative risk mitigation strategies are required
- Each configuration must be dose mapped
  - Someone else's protocol will not work!
- Radiation dose is always a range
  - The tighter the requirement, the harder it is to hit
  - Most regulations require 25 - 30KgY

Susceptibility of different model viruses to γ-irradiation

Inactivation data in serum



>4 log reduction**

** Calculated by Nims et al., 2011; Nims and Plavsic, 2012*

*** Recommended in ICH CPMP/ICH/295/95 and WHO TRS Nº924, 2004*

Effectiveness on Specific Viruses

- Examples are viruses of concern from the USDA Risk Assessment
- Blue tongue (BTV)
- Cache Valley (CVV)*
- Foot and Mouth (FMDV)
- Bovine Viral Diarrhea Virus (BVDV)

* While CVV is not on the USDA list it is a relatively new virus of concern

Relative Sensitivity to Irradiation

Virus	Family	Genome	Envelope	Particle Size (nm)	Irradiation Dose Used (kGy)	Log10 Reduction in Titer	Log10 Reduction with Heat
CVV	Bunyaviridae	ss-RNA, segmented	Yes	90–120	26–34	≥ 5.4	2.6
BVDV	Flaviviridae	ss-RNA	Yes	40–60	25–35	≥4.3	6.0
BTV	Reoviridae	ds-RNA, segmented	No	60–80	25–35	3.3	<1.0
FMDV*	Picornaviridae	ss-RNA	No	25 -37	25 -35	5.7	3.0

- SVDV a family member of FMDV has a log 10 reduction of 6

Summary

- Generally speaking, for three out of the four viruses it appears that Gamma provides around twice as much viral load reduction
- The outlier is BVDV where heat may be slightly more effective
- Given the variability of heat inactivation and its lower effectivity in viral reduction, heat inactivation of FBS is not an ISIA-recommended practice, unless it has been shown to be necessary for a specific cell culture application.
  - Heat inactivation may be required to inactivate complement in calf and adult bovine serum for use in some applications.

References available at
https://www.serumindustry.org/gamma-irradiation

This has been a high level look at these two post manufacturing treatment methods

Would you be interested in a more in depth review of gamma irradiation?

Thank You!