Comparison of The Efficacy of Barrier Treatments for Viral Risk Mitigation in Human vs. Bovine Serum

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NOTHING WORKS LIKE SERUM

INTERNATIONAL SERUM INDUSTRY ASSOCIATION

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Some Opening Remarks on Barrier Treatments

- I am a collector. Among other things, I collect viral inactivation data, and for years I have been interested in approaches (barrier treatments) for achieving viral inactivation in animal serum.
- Most of the data available for inactivating viruses in serum have been derived from studies on fetal bovine serum. I have presented these data previously in ISIA meetings and webinars.
- Very limited data are available for inactivating viruses in human serum. I will discuss my expectations and where possible, back these up with actual data.

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Why are Barrier Treatments needed for Bovine or Human Serum?

- Barrier treatments are intended to mitigate (not eliminate) the risk of introducing a viral contaminant into a geographical region (import concern) or a cell culture (user or biologics industry concern).
- The most common barrier treatments used for serum include gamma irradiation and heat inactivation.
- These barrier treatments each display dose-response efficacy curves. Higher doses may lead to serum damage, so increasing the dose is not always an option.

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Viral Risk in Non-Barrier-Treated Serum

- In non-treated serum, the risk of viral contamination is mitigated only by sourcing considerations (geography, oversight of abattoirs or blood collection sites) and testing for viruses in serum pools or product.
- Sampling considerations mean that insufficient risk mitigation is afforded by viral testing alone. Hence, the need for barrier treatment.

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Comparing Human vs. Bovine Serum

- Are there differences in viral risk for human vs. bovine serum?
  - The viruses of concern vary. While certain viruses represent risk for both types of serum, there are some significant differences which are relevant for our topic.
- In most respects, the characteristics (matrix effects) of human and bovine serum should be similar enough that we can extrapolate data from bovine serum to human serum.

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Viruses of concern in Bovine vs Human Serum

Virus Family	Virus of concern	
	Bovine serum	Human serum
Adenoviridae	bovine adenoviruses*†	
Bunyaviridae	Schmallenberg virus‡	
Coronavirus	bovine coronavirus‡	emerging coronaviruses
Flaviviridae	bovine viral diarrhea virus*†	hepatitis C virus§, West Nile virus§, Zika virus
Hepadnaviridae		hepatitis B virus§
Herpesviridae	bovine herpesvirus 1 (IBRV)‡	
Paramyxoviridae	shipping fever virus (PIV-3)‡	emerging paramyxoviruses
Parvoviridae	bovine parvovirus*†	parvovirus B19§
Picornaviridae		hepatitis A virus§
Pneumoviridae	bovine respiratory syncytial virus*†	
Polyomaviridae	bovine polyomavirus†	
Reoviridae	reovirus*†, bluetongue virus*†, bovine rotavirus‡	
Retroviridae	bovine leukosis virus‡	HIV-1,2§, HTLV-1,2§
Rhabdoviridae	rabies virus*†	

*Per 9 Code of Federal Regulations Part 113.47 Detection of extraneous viruses by the fluorescent antibody technique

†EMEA CPMP/BWP/1793/02. Note for Guidance on the Use of Bovine Serum in the Manufacture of Human Biological Medicinal Products

‡Viruses of USDA interest not already on the 9 CFR 113.47 list

§FDA CBER Requirements for Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use

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What are the Relevant Barrier Treatments for Serum?

- Gamma irradiation (deeply frozen serum)
- Heat treatment (liquid serum)
- Ultraviolet C irradiation (liquid serum, rarely used, I will address this in a future webinar)

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Direct and Indirect effects of Gamma Radiation

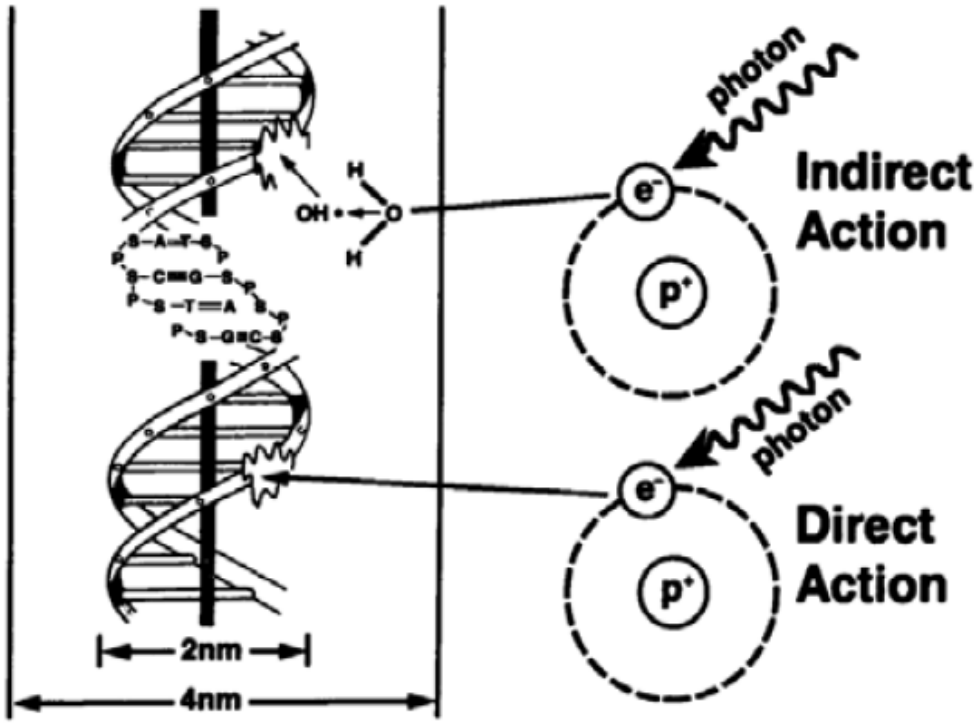

Fig. 2.2 Direct versus indirect action (Hall and Giaccia, 2006).

- Ambient temperature
- No scavengers
- Aqueous solutions
- Oxygenated

- Low temperature
- Scavengers
- Low oxygen

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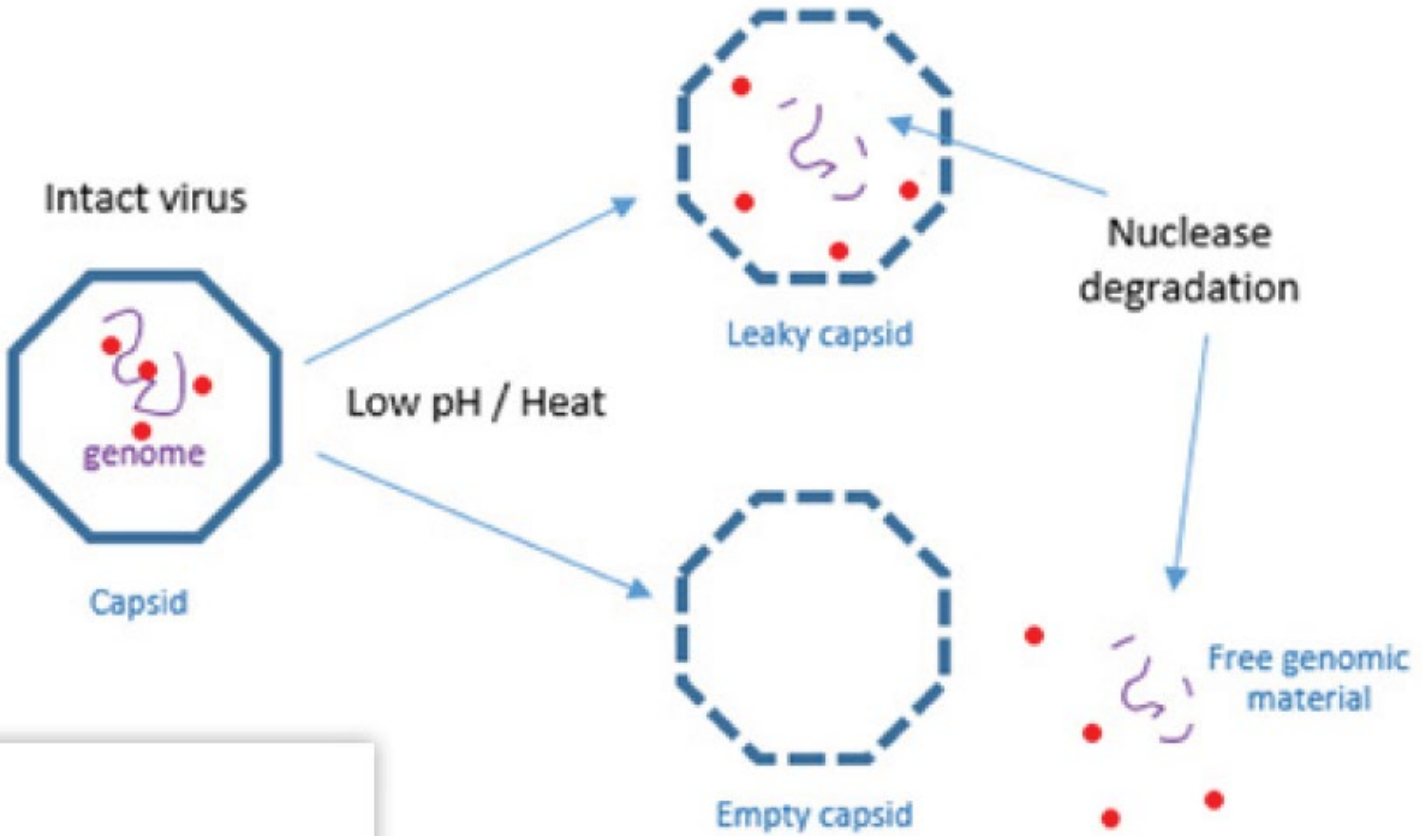
Postulated Mechanism of Heat Inactivation



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Virucidal Efficacy of Barrier Treatments

- The virucidal efficacies of barrier treatments depend on:
  - Fluence
    - Gamma irradiation: kGy (takes into account dose rate and time)
- Temperature and time
  - Heat inactivation: higher inactivation with greater time, temp
  - Gamma irradiation: higher inactivation with greater fluence
- Inactivation matrix, in particular:
  - Presence of scavenging protein, temperature, oxygenation (gamma)
  - Liquid vs. solid, presence of organic load (heat inactivation)

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Virucidal Efficacy of Barrier Treatments

- The virucidal efficacies of barrier treatments depend on:
  - The virus being inactivated
    - Particle size (gamma irradiation)
    - Capsid protein (heat inactivation)

  - Note that envelope status is not particularly important for determining the efficacy of these physical approaches.

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The Quick Answer on Mitigating Virus Risk in Human vs. Bovine Serum

- No differences expected in matrix effects alone
- No differences in gamma efficacy expected, given that similar fluences are applied at similar frozen temperature
- Heat treatment should be similarly effective for most viruses, but….is quite effective for human parvovirus B19 while not for bovine parvovirus)

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Expected Efficacy of Gamma Irradiation

Virus family	Bovine virus of concern	Human virus of concern	Expected efficacy of gamma irradiation		
			Inactivation (log_{10}/kGy)	Log_{10} Inactivation at 25 kGy	Virus tested
Adenoviridae	bovine adenoviruses		0.203	5.1	canine adenovirus
Bunyaviridae	Schmallenberg virus		0.40, 0.29	10, 7.3	Akabane virus, Ainovirus
Coronavirus	bovine coronavirus	emerging coronaviruses	0.63	>10	SARS-CoV-2
Flaviviridae	bovine viral diarrhea virus	hepatitis C virus, West Nile virus, Zika virus	0.198	5.0	bovine viral diarrhea virus
Hepadnaviridae		hepatitis B virus	No data		
Herpesviridae	bovine herpesvirus 1 (IBRV)		0.310	7.8	infectious bovine rhinotracheitis virus
Paramyxoviridae	shipping fever virus (PIV-3)	emerging paramyxoviruses	0.209	5.2	parainfluenza-3 virus
Parvoviridae	bovine parvovirus	parvovirus B19	0.071, 0.055	1.8, 1.4	mouse minute virus, porcine parvovirus
Picornaviridae		hepatitis A virus	0.19, 0.20	4.8, 5.0	foot & mouth disease virus, swine vesicular disease virus
Pneumoviridae	bovine respiratory syncytial virus		No data		
Polyomaviridae	bovine polyomavirus		0.041	1.0	SV-40
Reoviridae	reovirus, bluetongue virus, bovine rotavirus		0.194, 0.12	4.9, 3.0	reovirus-3, bluetongue virus
Retroviridae	bovine leukosis virus	HIV-1,2, HTLV-1,2	0.11	2.8	feline leukemia virus
Rhabdoviridae	rabies virus		0.340	8.5	bovine ephemeral fever virus

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Efficacy of Heat Inactivation

Virus family	Bovine virus of concern	Human virus of concern	Expected efficacy of heat inactivation (56°C, 30 min)		
			Estimated log_{10} bovine	Estimated log_{10} human	Virus tested
Adenoviridae	bovine adenoviruses		2.1	2.1	Human adenovirus 5*
Bunyaviridae	Schmallenberg virus		2.3	2.3	Rift Valley fever virus
Coronavirus	bovine coronavirus	emerging coronaviruses	4.7	4.7	animal coronaviruses and SARS-CoV-2
Flaviviridae	bovine viral diarrhea virus	hepatitis C virus, West Nile virus, Zika virus	6.0	4.4	bovine viral diarrhea virus*, hepatitis C virus†
Hepadnaviridae		hepatitis B virus	0.5	0.5	hepatitis B virus
Herpesviridae	bovine herpesvirus 1 (IBRV)		1.7	1.7	herpes simplex-1*
Paramyxoviridae	shipping fever virus (PIV-3)	emerging paramyxoviruses	9.7	4.1	Newcastle disease virus, Nipah virus†
Parvoviridae	bovine parvovirus	parvovirus B19	<1	>10	Bovine parvovirus, human parvovirus B19
Picornaviridae		hepatitis A virus	3.0	3.0	poliovirus 1*
Pneumoviridae	bovine respiratory syncytial virus		>10	>10	respiratory syncytial virus
Polyomaviridae	bovine polyomavirus		0.5	0.5	SV-40
Reoviridae	reovirus, bluetongue virus, bovine rotavirus		2	<1	bovine rotavirus, reovirus-3,
Retroviridae	bovine leukosis virus	HIV-1,2, HTLV-1,2	>10	>10	HTLV-III
Rhabdoviridae	rabies virus		>10	>10	rabies virus, vesicular stomatitis virus

*Values obtained in bovine serum

†Values obtained in human serum

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Some Comments about The Comparative Efficacy Tables

- The efficacy of gamma irradiation was assessed at the low end of the typical serum irradiation dose (25 kGy). Most of the data shown are for irradiation at very low temperature in bovine serum.
- Not expected to be very effective for: polyomaviruses or parvoviruses even at 45 kGy

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Some Comments about The Comparative Efficacy Tables

- The efficacy of heat is provided at conditions typically used for heat-inactivating serum (56°C for 30 minutes). The data shown are for heating in liquids, and in some cases, human or bovine serum.
- Not expected to be very effective for: polyomaviruses, bovine parvovirus, reoviruses, adenoviruses, bunyaviruses
- But, importantly, human parvovirus B19 is very susceptible to this heat treatment!

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Conclusions

- Gamma irradiation is relied on to mitigate the risk of most of the viruses of concern from the USDA, World Health Organization, and biologics manufacturing points of view. It is not perfect, though.
- Few remaining viruses that will likely survive gamma irradiation are the parvoviruses and polyomaviruses.
- These resistant viruses do not appear to be of great import concern.

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Conclusions

- Heat inactivation at 56°C, 30 min. could mitigate the risk of some of the viruses of concern from the USDA, World Health Organization, and biologics manufacturing points of view. Some of the viruses of concern that may survive heat treatment are the same viruses that survive gamma treatment: polyomaviruses, bovine parvovirus, herpesviruses, adenoviruses, reoviruses, and bunyaviruses.

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Recommendations

- Gamma irradiation is the best barrier treatment for serum, both bovine and human. Heat inactivation is more effective than most people think, but the list of resistant virus types is longer in the case of heat. Human parvovirus B19, a virus of concern for human serum, is very susceptible to heat (unlike the animal parvoviruses).
- Because of this, I advocate using both heat inactivation and gamma irradiation for barrier treating human serum.

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Questions?

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