Inactivation of Viruses

An Introduction to the Series

by Gail Sofer

During the past five years or so, FDA has been more stringent about virus clearance studies. Although rare, cases exist of viral contamination of cell substrates, production facilities, and products. As a result, demand for more effective initial virus clearance studies is increasing. The intent is, of course, to ensure patient safety. The European Union draft on the manufacture of products intended for clinical trials states that "Virus inactivation/removal and removal of other impurities of biological origin should be no less rigorous than for licensed products" (1).

Applying a virus inactivation method during production can alleviate some concerns about potentially infectious, adventitious, or endogenous agents. Virus inactivation processes are usually less susceptible to minor changes than removal steps are — that is, virus inactivation processes are said to be robust. FDA's "Points to Consider" on monoclonal antibody products for human use says that robust viral inactivation processes include solvent/ detergent, low pH, and heat treatments (2).

Controlling Critical Parameters

Although virus inactivation treatments are often robust, many factors have the potential to affect a method's efficiency. The guideline from the International Conference on Harmonisation (ICH) states, "Virus inactivation is not a simple, first-order reaction and is usually more complex, with a fast 'phase 1' and a slow 'phase 2'" (3). Time is an obvious critical parameters to control during virus inactivation. There are many other variables that are critical to ensure that virus inactivation is, indeed, robust.

- **Virus stock and titer** are important elements that can influence the log reduction result from a viral inactivation study.
- **Variability in the test article** can profoundly influence the outcome and can inhibit or interfere with an assay system. Ranges for impurities, protein concentrations, and additives should be defined so that they don't affect log reduction values.
- **Temperature** usually has a significant effect on inactivation kinetics.
- **Scale-down accuracy** is another important element when designing virus inactivation studies that will need to provide reliable data when translated to manufacturing scale.

Variability Can Affect Results

Viruses, treatments, and products are idiosyncratic — each requires specific experience, evaluation, and testing to understand the range of acceptable results and the critical parameters that can affect those data. Some examples of the effects of variability include the following.

pH. When evaluating the effectiveness of low pH on virus inactivation, accurate scale-down and mixing are critical parameters; for instance, comparing an overhead impeller stirrer to a stirring bar can be problematic because inactivation by pH can vary with different stirring efficiencies, and shear forces can affect the inactivation of some viruses. The consistency of the test article can be an important variable. Both the pH and treatment time must be monitored with suitably calibrated equipment. In one study, pH 7.5 was found to be more effective than pH 6.4 for virus inactivation in a 5% plasma protein solution, but for a 5% albumin solution pH 6.4 was more effective than pH 6.9 or pH 7.4. In this case, the excipients were found to influence the pH inactivation (4).

Heat. Virus inactivation by heat can be effective. Monitoring the temperature and treatement time is essential to ensuring confidence in the validity of a viral inactivation study. For dry heat, moisture content is also critical. Dry heat has been added to some processes to enhance viral safety. However, for some viruses (such as porcine parvovirus), consistent survival after lyophilization can occur even at temperatures between 95°C and 100°C (5).

Solvents/detergents. Several references in the literature describe the many variations that are observed when detergents are applied as viral inactivation agents. Solvent/detergent methods certainly enhance the confidence in the safety of plasma products, but some detergents alone are not very effective. Detergent concentration and the type of detergent used can alter the effectiveness of the inactivation. The contact time, temperature, and test article properties are all important parameters.

On the Horizon

The ICH guideline on viral safety says, "For each production step assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal." Today, polymerase chain reaction (PCR) tests combined with infectivity assays enable us to better understand viral clearance mechanisms (see Table 1). Technology will, hopefully, help us understand virus stabilization effects of resins and filters that might affect overall safety assessments.

New inactivation methods are being investigated to further enhance viral safety. The motivation behind these investigations is concern about bioterrorism and the rapid transport of viral agents by the traveling public. Conversely, the more we can detect, the more concern is raised, and sensitive methods, such as PCR, allow detection of viruses that might not present a safety risk. New cell culture methods for specific viral agents and more sensitive detection methods provide us with better technologies for assessing and understanding virus inactivation.

References

(1) Enterprise Directorate-General, *Good Manufacturing Practices: Manufacture of Investigational Medicinal Products*, Vol. 4, Annex 13, Draft 1, (European Commission, Brussels, Belgium, November 2001).

(2) CBER, *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* (FDA, Rockville, MD, 28 February 1997).

(3) ICH Steering Committee, *ICH Harmonised Tripartite Guideline: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (ICH, Geneva, 5 March 1997).

(4) Rosenthal, S. et al., "Pasteurization of Cohn Fraction V Products: Effect of pH on Viral Inactivation," poster presentation at the PDA/FDA Viral Clearance Forum, Bethesda, MD, October 2001 and personal communication with M. Korneyeva, Bayer Corporation.

(5) BioReliance databases (BioReliance Corporation, Rockville, MD). ***BPI***

Table 1. The inactivation of X-Mulv at a low pH and an infectivity assay ($TCID_{50}$) with quantitative PCR (Q-PCR) clears the infectivity, but the viral sequence remains.

Treatment for Various Lengths of Time	Total Virus (log_{10})		Clearance (log_{10})	
	$TCID_{50}$	Q-PCR	$TCID_{50}$	Q-PCR
Low pH, T= 0 min.	5.71±0.24	10.40±0.09		
Low pH, T=30 min.	≤3.67	10.29±0.08		
Low pH, T=60 min.	≤2.66	10.23±0.03	≥4.39±0.32	0.17±0.03

Virus Charts. All enveloped and nonenveloped viruses in this series, with abbreviations, and reference numbers that refer to that virus (Part #s are in color[a])

Enveloped Virus

Name	Abbreviation	Reference[a]
Avian influenza virus		8
Bovine herpes virus	BHV	19,23,50, 5,6,18,20
Bovine immunodeficiency virus	BIV	5
Bovine leukemia virus	BLV	46
Bovine viral diarrhea virus	BVDV	15, 20,22,23,25,26, 12,19,23,27, 29,30,39,45,50,53,58,59, 5,18,20, 23,26,32,38,43,8,13,14,17,46, 27
Chikungunha virus	CHV	20
Classical swine virus		5
Cytomegalovirus	CMV	15.20, 7,20,23,25,26,37–39, 21,22 32,46,47, 41, 18,47, 7
Dengue virus		36
Duck hepatitis B virus	DHBV	15, 7,20,23,25,26,37–39, 21,22,46 47,53, 11, 16
Eastern equine encephalomyelitis		46
Ectromelia virus (poxvirus)	ECT	22
Equine arteritis virus	EAV	18
Feline leukemia virus	FeLV	9,18, 19, 17
Feline sarcoma virus	FeSV	6,7
Foot and mouth disease virus	FMD	17,41,42,46
Friend murine leukemia virus	Fr-MuLV	7
Hepatitis B virus	HBV	38, 15,46–48,52–56, 17,22, 14
Hepatitis C virus	HCV	15,20,46,47,52–56, 8,17,22,43
Hepatitis D virus	HDV	47
Herpes simplex virus	HSV	2–5,22, 20,23,30,41,42, 5,27,30, 32,36,37,46–48,56, 5,6,9,12,23,35, 41, 14,18,26
Hog cholera virus		11
Human coronavirus		31
Human immunodeficiency virus	HIV	2,6,10–19,23–25, 6,14–17,19,20, 21,23–26,28–33,39, 5,9,12,15, 17–20,23,25,27,30–33,36,37, 41–47, 50–59, 4–8,10,11,16,18, 20–24,27,33,38,41,42, 6,7,14,15, 18–21,39,44, 9,11,13,18,23,31,33
Infectious bronchitis virus		8
infectious laryngotracheitis virus		8
Influenza		5,6, 44
Junin virus		19
Lassa fever virus		44
Measles virus	MV	33,37
Mink cell focus virus	MCF	22
Mumps		20,
Murine leukemia virus	MuLV	47, 27
Newcastle disease virus		46, 8
Parainfluenza	PI	47, 8,17,22
Pichinde virus		44
Pseudorabies virus	PRV	16, 4,5,22,30,35,36,42, 6,12,17, 28,23,25,36,37,45,46,51,58,59, 18,26,27,38,39,8,24,46, 27
Rauscher MuLV, ecotropic		47
Respiratory syncytial virus	RSV	7,38, 32,33
Retrovirus		6, 14
Rous sarcoma virus		6,7
Semliki forest virus	SFV	5, 5,6,11,18,20,35,38–41, 16
Sendai virus	SEN	47,56
Simian immunodeficiency virus	SIV	14, 20, 5,6,18,20,27, 18,20,31
Simian sarcoma virus	SSV	6,7
Sindbis virus	SIN	16, 6,14,16,17,27,28, 5,9,10,19,20, 36,45–48,50–54,56,57,60, 5,6,27,
Suid herpes virus	SHV	5,6,9,20
Swine vesicular disease virus	SVDV	11, 20
Tick-bourne encephalitis virus	TBEV	17,18,23,25,27,29,30, 23
Vaccinia virus		21,22, 9, 5,20,32, 35, 33
Venezuelan equine encephalomyelitis	VEE	47,56, 43
Vesicular stomatitis virus	VSV	7,25, 4–8,10–14,16,17,19,22,23, 27–30,33–36,40,41, 5,12,20,32, 44–47,49,50,52–54, 56,57, 5,6,11, 12,17,18,20,27–31,33,34,40,41,12
Visna virus		32, 14,20,26,29,35,46,
Xenotropic murine leukemia virus	XmuLV	47, 22–24
Yellow fever virus	YFV	27,29,30, 23
West Nile virus	WNV	5

Nonenveloped Virus

Name	Abbreviation	Reference[a]
Adenovirus	ADV	4–6,11,47,48, 31
Avian reovirus		8
Baculovirus		34
Blue tongue virus	BTV	17, 8,11,46
Bovine parvovirus	BPV	5,12, 8,17
Calici virus		5,6,11,18
Canine adenovirus		10
Coxsackie virus	CV	36,37, 31
Canine parvovirus	CPV	5,7, 27
Echovirus		17
Enteroviruses		17
Human rhinovirus	ECHO	20
Encephalomyo-carditis virus	EMC	6,14,19,23, 19,23,44,5,6, 11,26,27,28, 33,34
Equine rhinovirus	ERV	18,24,
Feline calicivirus	FCV	19,20, 22
Hepatitis A virus	HAV	6,7,11–13,16, 17,22–27,36–40,17–20,23, 25,27,30–33, 36,37,41–47, 50–59, 5,11, 33,34, 12,40. 40, 21,33
Infectious bovine rhinotracheitis virus	IBRV	48,17,22,46
Infectious bursal disease virus		8
Lambda phage		25
Minute virus of mice	MVM	17,23,25,
Murine encephalo-myelitis virus	MEV	5,6,18,20
Murine minute virus	MMV	9,11,16
Parvovirus		34
Parvovirus B19	PV-B19	8,11,28,29,36
Picornavirus		34
Poliovirus		2–5,8,15,16, 41,43,13,24, 27,30,32,36, 37,5,6,23,35, 2,15,17,31,33
Porcine circovirus	PCV	12
Porcine enterovirus	PEV	13
Porcine parvovirus	PPV	22, 6,19,32,5, 6,11,18,20, 26,33,34,10, 8,13,17,34,46
Reovirus	Reo	12 5,30, 8,17
Rotavirus		8, 30,45
Simian virus	SV-40	22, 12,36,5,6, 11,18,20

[a]References are color-coded to the Part number in this series, which also provides information on the test article: Part 1: Skin, Bone, and Cells (References on page 10); Part 2: Red Blood Cells and Platelets (References on page 14); Part 3a: Plasma and Plasma Products (Heat and Solvent/Detergent Treatments) (References on page 23); Part 3b is Plasma and Plasma Products (Treatments Other than Heat and Solvent/Detergent Treatments) (References on page 28); Part 4 is Culture Media, Biotechnology Products, and Vaccines (References on page 33); Part 5 is Disinfection (References on page 36).
[b]Fourth derivative UV spectroscopy.
[c]Light scattering and ultracentrifugation.
[d]Western blot, SDS-PAGE gels (silver stained).