Modular Retrovirus Clearance in Support of Clinical Development

Bio-product Research & Development,

Eli Lilly and Company

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General Expectations for Viral Clearance

Category	Clinical Trial Application	Marketing Application
Model Viruses	1 to 2	3 to 5
Virus Partitioning	not required	required
Inactivation Kinetics	not required	required
Robustness Studies & Demonstration of Effective Control in Production	not required	required
Virus Carryover Studies	not required	required
Resin Life Studies	not required	Yes
Batches Tested for RVLP	not specified and number from a single batch is acceptable	≥ 3
Modular/Generic Claim	Yes	No/Not Yet

Generic and Modular Virus Clearance

Per FDA "*Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use*" (1997)

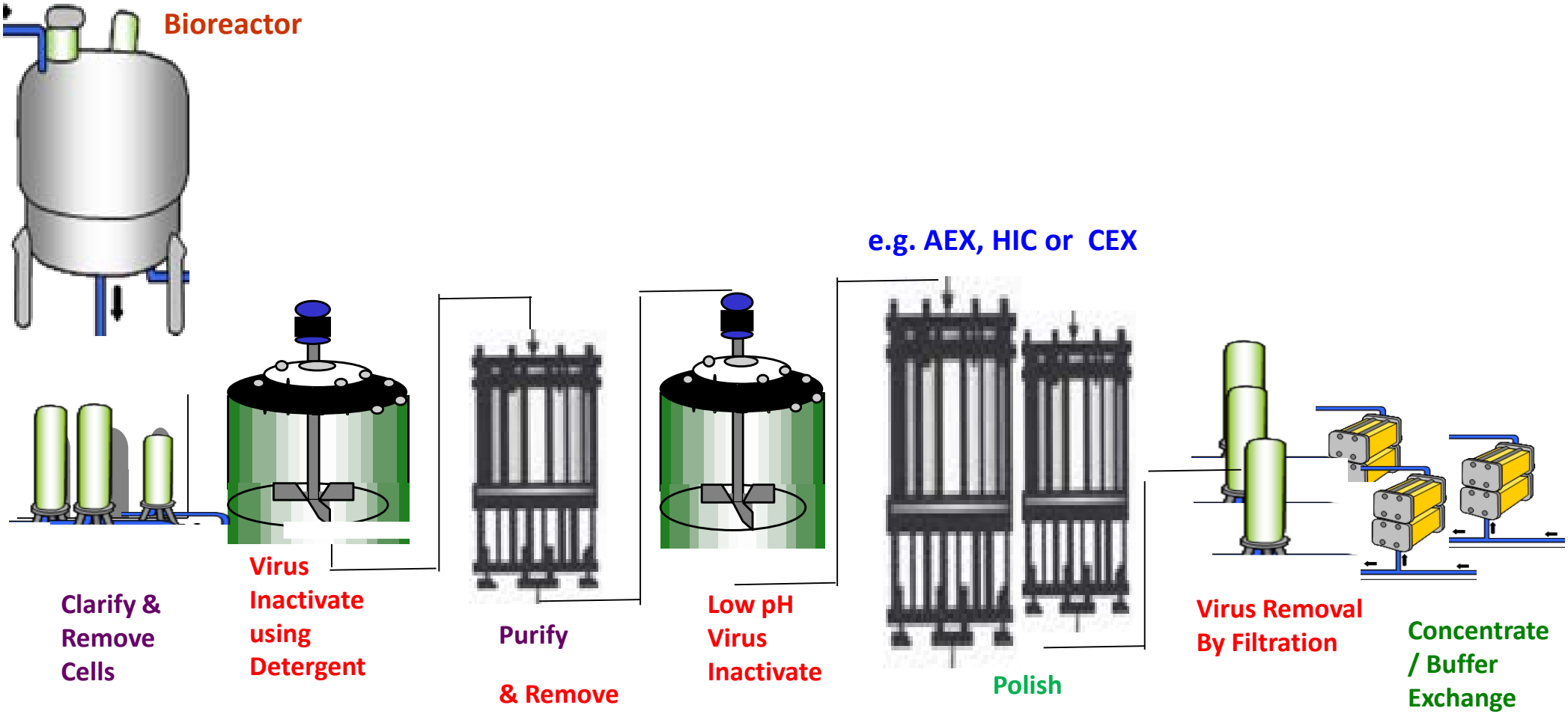
- "A generic clearance study is one in which virus removal and inactivation is demonstrated for several steps in the purification process of a model antibody. These data may then be ***extrapolated to other antibodies following the same purification and virus removal/inactivation scheme as the model antibody***"
- "A modular clearance study is one that ***demonstrate virus removal or inactivation of individual step during the purification process*** (column chromatography, filtration, pasteurization, solvent/detergent, low pH, etc). Each module in the purification may be studied independently of the other modules. Different model mAb may be used to demonstrate viral clearance in different modules, if necessary. If the purification process of a product mAb differs at any of the virus removal or inactivation modules from the model mAb, this module must be studied independently from the model. The other, identical modules in the procedure may be extrapolated to the product mAb."

Regulatory Expectations

Per EMA *"Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products"* (2008)

- "In the event that a manufacturer is developing similar types of products by established and ***well characterized*** procedures, virus reduction data derived for these other products might be applicable to the new product for an equivalent processing step."
- "In order to make use of data from such a step, the step should have been carefully evaluated, including a ***thorough study of the process parameters*** that affect virus reduction."
- "A rationale should be provided why prior ***in-house*** data can be applied to the new product, e.g. referring to viral reduction data of a particular process step would be possible when the product intermediate at the stage before such a step has comparable biochemical properties and is purified by identical methods. The manufacturer should provide a critical analysis of the manufacturing step for which in-house data will be applied and on the composition of the respective product intermediate"

Schematic Downstream Processes



Dedicated Virus Reduction Unit Operations at Lilly

Unit Operation	Positioning	Reduction Spectrum	Mechanism	Impact on Product Quality	Purification Burden
Detergent (Triton X-100)	Immediately after primary recovery by centrifugation and filtration	Selective (enveloped viruses)	Disruption of structural integrity critical for virus infectivity	No	Yes
Low pH	Immediately after Protein A affinity chromatography	Selective	Denaturation of viral proteins essential for virus viability	Yes	No
Filtration	After the final polishing chromatography	Broad range	Size based retention	No	No

Lab Scale Triton X-100 Inactivation Conditions

Parameter	Range	Rationale
Triton X-100 Concentration (%w/v)	0.45	Effectiveness and kinetics of virus inactivation by detergent is a concentration dependent reaction
Temperature (°C)	15 - 25	Higher temperature favor the inactivation reaction
Total Protein Concentration (mg/mL)	4.0 - 20.1	Protein concentration could potentially interfere with the inactivation reaction
Incubation Time (min)	60	Virus inactivation by Triton X-100 is time dependent as a chemical reaction

In-house Triton X-100 Inactivation Data

Description	Mol-1	Mol-2	Mol-3	Mole-4	Mol-5	Mol-6	Mol-7	Mol-8	Mol-9	
	IgG_4	IgG_4	IgG_4	IgG_4	IgG_4	IgG_4	IgG_1	Fusion	Protein	
Protein (mg/mL)	13.2	11.4	15.3	8.6	20.1	16.3	8.0	5.4	4.0	
Inactivation Kinetics as Measured by Log_{10} Reduction Factor (LRF)										
Spiked Load	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Initial (T=0 min)	≥2.61	≥2.72	2.75	≥3.18	≥3.76	≥2.87	≥3.74	≥3.64	≥3.09	≥2.83
T=10 min	≥2.61	≥2.72	≥2.85	≥3.18	≥3.76	≥2.87	≥3.74	≥3.64	≥3.09	≥2.83
T=30 min	≥2.61	≥2.72	≥2.85	≥3.18	≥3.76	≥2.87	≥3.74	≥3.64	≥3.09	≥2.83
T= 60 min	≥2.61	≥2.72	≥2.85	Not Done	Not Done	≥2.87	≥3.74	Not Done	≥3.09	≥2.83
T=60 min (LT)	**≥4.67**	**≥4.68**	**≥4.80**	**≥4.85**	**≥5.73**	**≥4.83**	**≥5.70**	**5.14**	**≥5.00**	**≥4.74**
Hold Control (T=60 min)	0.12	0.00	0.50	-0.03	0.20	0.38	0.37	-0.04	-0.09	-0.52

Conclusions: *Robust murine retrovirus inactivation is consistently achieved under the experimental conditions described. In all cases except one, retroviruses were inactivated to below the limit of detection. Therefore, it's possible that modular clearance can be claimed for future molecules provided specific criteria are adequately met.*

Modular Claim for Triton X-100 Inactivation

Based on the in-house data from multiple (9) molecules, **5.14 $\log_{10}$** retrovirus reduction by Triton X-100 is modularly claimed in support of clinical development provided that the unit operation is positioned immediately after the removal of cells and following criteria are met in manufacturing.

GMP Production	Criteria
Production Cell Line	CHO
Triton X-100 Concentration (w/v)	≥ 0.45 (targeting 0.55%)
Temperature (°C)	15 - 25
Incubation Time (min)	≥ 60

We also have in-house data demonstrating that Lipid, DNA, and total protein concentrations do not affect the effectiveness of retrovirus inactivation by Triton X-100 under the manufacture conditions defined.

Lab Scale Low pH Inactivation Conditions

Parameter	Range	Rationale
pH	3.65 ± 0.45	Inactivation is directly linked to pH
Temperature (°C)	15 - 25	Higher temperature, faster the inactivation reaction
Product Concentration (mg/mL)	5.0 – 23.6	Protein concentration could potentially interfere with the inactivation reaction
Incubation Time (min)	Up to 120 min	Virus inactivation by low pH is time dependent as a chemical reaction

In-house Low pH Inactivation Data

Description	Mol-1			Mol-2			Mol-3		
	IgG_4			Fusion Protein			IgG_1		
Product (mg/mL)	9.6	11.8	15.6	5.9	5.9	6.6	18.8	18.8	23.6[#]
Inactivation Kinetics as Measured by Log_{10} Reduction Factor (LRF)									
Spiked Load	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Initial (T=0 min)	3.27	2.99	≥5.36	≥4.69	≥4.49	≥4.23	≥4.91	≥5.20	3.41
T=5	Not Done	Not Done	Not Done	≥4.69	≥4.64	≥4.23	≥4.91	≥5.20	4.80
T=10	Not Done	≥5.30	≥5.36	≥4.69	≥4.49	≥4.23	≥4.91	≥5.20	5.50
T=30 min	≥4.11	≥5.30	≥5.36	≥4.69	≥4.49	≥4.23	≥4.91	≥5.20	≥5.24
T=60 min	≥4.11	≥5.30	≥5.36	**≥5.96**	**≥5.86**	**≥5.90**	**≥4.91***	**≥7.16**	**6.16**
T=120 min	**≥5.11**	**≥7.15**	**≥7.21**	[*] No large volume testing was performed; [#] Study was performed at lower temperature					
Hold Control	0.53	0.50	0.17	0.47	0.49	0.05	0.00	0.50	0.35

- *Inactivation by low pH is rapid. Virus is generally not detectable at the first time point (5 or 10 min);*
- *Product concentrations have little impact on inactivation kinetics or LRF when everything else remains constant*

Complete Low pH Inactivation Data Set

Molecule	Description	Retrovirus LRF Demonstrated
1	mAb	≥4.47
2	mAb	≥4.67
3	mAb	≥6.83
4	mAb	≥5.11; ≥7.15; ≥7.21
5	mAb	**5.79**
6	mAb	≥6.15
7	mAb	>6.39
6	mAb	>6.41
9	mAb	≥5.83; ≥5.23
10	mAb	≥6.28
11	mAb	≥4.18
12	mAb	>5.16
13	mAb	**6.01**
14	Bispecific mAb	**7.03**
15	Fusion	>5.96; >5.86; >5.90
16	mAb	≥4.91; ≥7.16; **6.16**

Under the defined conditions, in-house data obtained from 16 different molecules have shown that low pH treatment provides consistent and robust retrovirus inactivation. In most cases, model retroviruses were inactivated to below the limit of detection. In *four* individual experiments, residual surviving viruses were indeed detected. However, the lowest LRF achieved among these four studies were **5.79**.

Modular Claim for Low pH Inactivation

Based on the in-house data from multiple (16) molecules, **5.79 $\log_{10}$** retrovirus reduction by low pH treatment is modularly claimed in support of clinical development provided that the unit operation is positioned immediately after the Protein A affinity chromatography and following criterial are met in manufacturing.

GMP Production	Criteria
Production Cell Line	CHO
Preceding Unit Operation	Protein A Affinity Chromatography
pH	≤3.60
Temperature (°C)	≥18
Incubation Time (min)	≥120
Buffer System	Citrate

Lab Scale Viral Filtration Conditions

Parameter	Range
Virus Filter	Planova 20N
Temperature (°C)	15 - 25
Pressure (PSIG)	12 - 16
Product Concentration	3.09 – 12.97
Throughput (g/m^2)	571 - 2225

In-house Planova 20N Filtration Data

	Mol-1	Mol-2	Mol-3	Mol-4	Mol-5	Mol-6	Mol-7	Mol-8	Mol-9
Description	mAb	mAb	mAb	Protein	Fusion	mAb	mAb	Bispedific	Fusion
Product (mg/mL)	5.71	12.97	7.22	4.98	9.58	4.94	7.32	3.09	11.12
Preceding Polishing Chromatography	A	B	B	C	D	B	B	E	E
Inactivation Kinetics as Measured by Log_{10} Reduction Factor (LRF)									
Throughput (L/m^2)	104	150	201	205	200	164	187	201	**200**
Throughput (g/m^2)	594	1947	1449	1018	1919	808	1365	622	**2225**
Flux Decay (%)	9.8	31.7	65.3-88.4	5.5-13.5	28.6	94.4	30.0-43.1	29.2	**80**
Retrovirus LRF Achieved	**≥4.67**	**≥6.93**	**≥6.46**	**≥6.25**	**≥5.00**	**≥6.00**	**≥5.55**	**≥6.16**	**≥6.13**
Parvovirus LRF Achieved	**7.15/5.63**	**3.41/3.94**	**3.50/3.25**	**4.25/4.88**	**3.04/4.04**	**≥5.75/≥6.01**	**2.86/3.73**	**3.47/3.56**	**3.73/4.87**

Conclusions: Reduction of parvovirus varies among processes. However, retroviruses were always removed to below the limit of detection without any exception.

Historical Data from Industry

Retrovirus Removal Studies by Parvovirus Filters

Company	Number of Biological Entities Evaluated
Human Genome Science (now GSK)	8
Novartis	13
Pfizer	18
Wyeth	5
Amgen	25
Eli Lilly	12
Genentech	14
Boehringer Ingelheim	14

Source: Proceedings of the 2009 Viral Clearance symposium. 2010. Devel. Biol. Volume 133, pp 77-91

Facts

- Retrovirus breakthrough of parvovirus filters has not been seen.
- Parvovirus breakthrough is not uncommon across all major brands. However, severity may vary.
- Large body of data indicates that parvovirus filters can reliably and consistently remove retroviruses to below the limit of detection (100% retention).

Relevant Questions

- Could retrovirus pass through parvovirus filters under the conditions when parvovirus breakthrough is occurring?
- What specific LRF value could one claim for modular retrovirus clearance?
  1. *Claim the LRF achieved with parvovirus such as PPV or MMV*
  2. *Use surrogate model virus (e.g. phage) that is larger than parvovirus but smaller than retrovirus, thus making it possible claim a higher LRF*
  3. *Use the historical in-house retrovirus LRF achieved (e.g. XMuLV)*

Co-spiking Experiments

- To determine whether retrovirus breakthrough occur under conditions when parvovirus breakthrough is taking place.
- To provide scientific basis to claim modular retrovirus clearance and to justify how many log reduction factors to claim based on in-house data set.

Results of Co-spiking Study

Molecule	*Retrovirus (XMuLV) LRF*		*Parvovirus (PPV) LRF*	
	Run 1	***Run 2***	***Run 1***	***Run 2***
Molecule 1	≥ 5.73 ± 0.35	≥ 4.65 ± 0.36	4.71 ± 0.32	≥ 4.84 ± 0.56
Molecule 2	≥ 7.45 ± 0.30	≥ 7.37 ± 0.17	3.97 ± 0.41	3.71 ± 0.40
Molecule 3	≥ 5.83 ± 0.30	≥ 6.10 ± 0.34	4.47 ± 0.47	5.15 ± 0.54
Molecule 5	≥ 6.17 ± 0.41	≥ 6.17 ± 0.34	4.45 ± 0.49	4.76 ± 0.55
Molecule 10	≥ 6.17 ± 0.35	≥ 6.17 ± 0.30	5.19 ± 0.77	≥ 5.11 ± 0.36
Molecule 11	≥ 6.44 ± 0.34	≥ 6.52 ± 0.30	3.59 ± 0.43	4.20 ± 0.50

Criteria for Modular Claim

Based on in-house data from *9* processes/molecules and results from the co-spiking study, an LRF of ≥6.93 for retrovirus clearance is justified to support clinical trial applications when the following conditions are met:

- Biologics are made in CHO cells
- Planova 20N filters are used in production
- Trans-membrane pressure is controlled at 12-16 psig
- Pass the post-use filter integrity test.

Viral Clearance for Clinical Trials

Unit Operation	Retrovirus	Parvovirus
DVI	Modular (LRF 5.14)	**Not Evaluated**
Chromatography	Not Evaluated	**Two runs**
Low pH	Modular (LRF 5.79)	**Not Evaluated**
Viral Filtration	Modular (LRF ≥6.93)	**Two runs**
Total Modular LRF Claimed	**≥ 17.9**	**Not Applicable**

Summary

Based on extensive in-house datasets, Lilly has provided compelling scientific basis to justify modular retrovirus clearance for all three dedicated unit operations in our downstream purification processes to support clinical development (IND/CTA) when predetermined specific criteria are met.

A Discussion Point

Can the same modular approach described be used to support marketing application?