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Inline Spiking Method for Validating Continuous Virus Filtration

Herb Lutz (principal consulting engineer BSN process solutions)

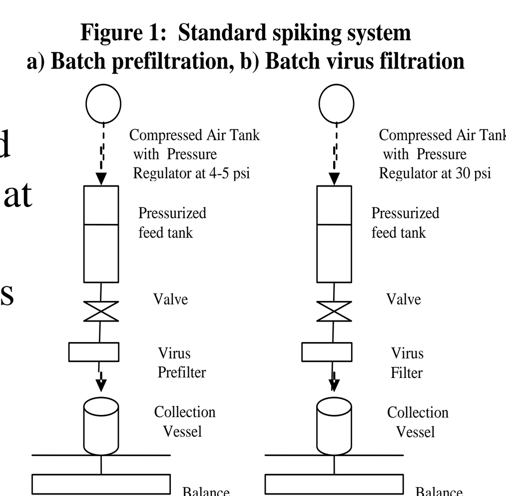
Introduction

Conventional virus filtration (VF) processes involve batch processing a uniform feed at uniform conditions (e.g. constant pressure). Continuous VF processes can involve an elutiion protein peak with a salt gradient under non-uniform pressure where standard spiking methodologies do not represent the manufacturing process as required by the ICH Q5A virus validation guidelines.

This paper describes the development, qualification, and implementation of an inline spiking methodology that is suitable for validating continuous processes.

Standard spiking

Figure 1: Standard spiking system
a) Batch prefiltration, b) Batch virus filtration

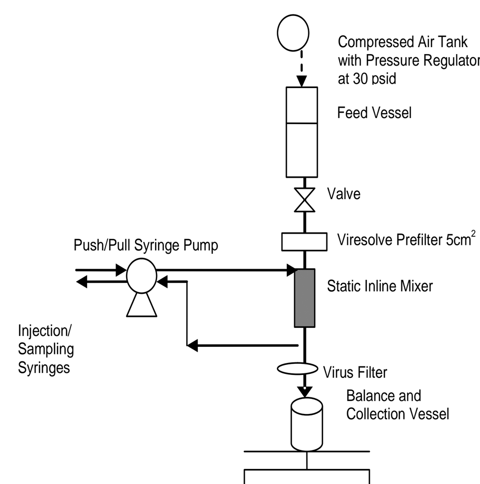


Batch prefiltration
Flush prefilter with water and buffer. Perform prefiltration at constant pressure. Filter at typical process times of ~2hrs to ensure comparable residence times for plugging agent removal

Batch virus filtration
Add virus spike to batch prefiltered product. Filter spiked solution with 0.2 or 0.45mm membranes. Perform virus filtration, sample filtrate weight at regular time points

Inline spiking

Batch prefilter-> spike-> virus filter



Flush filters with water and buffer. Add 0.2 mm filtered protein solution to the feed vessel and 0.2 mm filtered spiking solution to the feed syringe pump. Pressurize feed vessel, open valves. Start syringe pump to continuously inject spike, mix with refiltered feed, sample, and feed to virus filter. Measure filtrate weight vs time & calculate flow rate. As the virus filter plugs and flow drops, slow down the syringe pump to maintain % spiking ratio

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Inline Qualification with salt spike

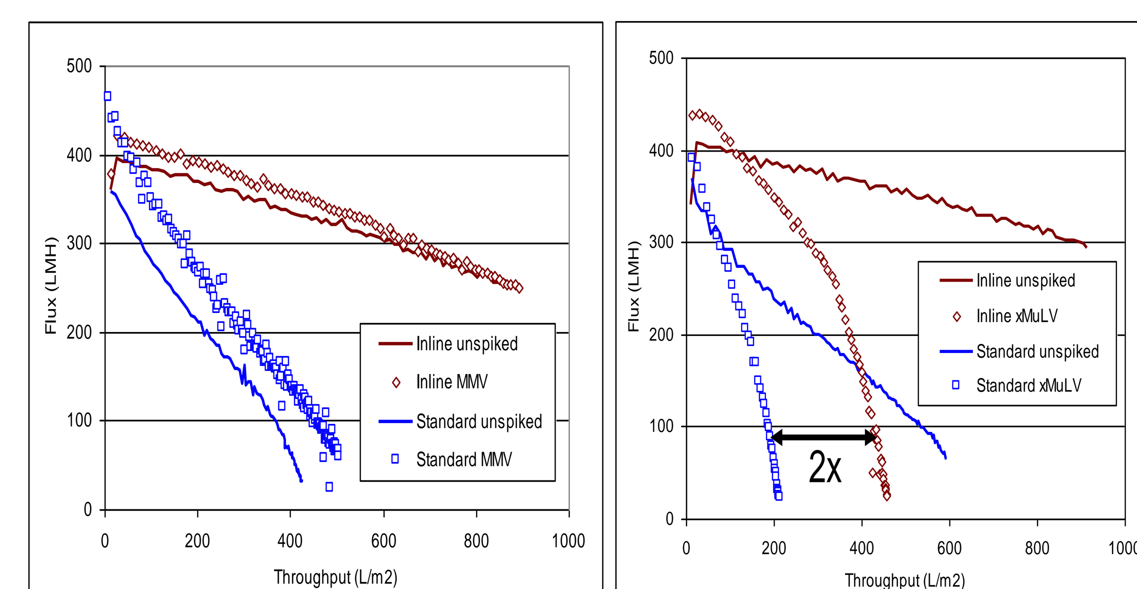
Salt spike improves sensitivity of concentration measurements: 2x difference in salt concentration (conductivity) corresponds to a 0.3 difference in $\log_{10}$ scales.

Run #	% Spike flow to filtrate flow	Spike (M)	Predicted Filtrate/Sample Syringe (M)	Sample Syringe (M)	Filtrate (M)
1	5%	0.68	0.028	0.024	0.028
2	5%	0.68	0.029	0.025	0.029
3	5%	0.68	0.026	0.024	0.026
4	10%	0.68	0.070	0.063	0.064
5	10%	0.68	0.069	0.057	0.064
6	10%	0.68	0.068	0.064	0.065

Run to run variability was <6%. Sample syringe titer can be used as feed pool titer. Sample syringe and filtrate were used within 14% (0.07 log10 units or ~0 LRV). The predicted filtrate/sample syringe titer within 9% (Hold control ~0 LRV). Well mixed solution so sample syringe is representative of cross section. Mass balance closes & holdup volumes are negligible

Inline qualification with prefiltration

mAb2 and Viresolve™ Pro spiked with 5% MMV or 5% xMuLV



MMV plugging due to protein. xMuLV plugging due to protein+spike. 2-4x capacity increases with inline spiking (even with xMuLV spike)

Run Description	Spike Titer $\log(TCID_{50}/mL)$	Predicted Sample Titer $\log(TCID_{50}/mL)$	Actual Sample Titer $\log(TCID_{50}/mL)$	LRV
MMV-inline	6.68	5.38	5.30	≥4.42
MMV-standard	--	--	5.68	≥4.80
xMuLV-inline	6.10	4.80	4.60	≥3.42
xMuLV-standard	--	--	4.48	≥3.30

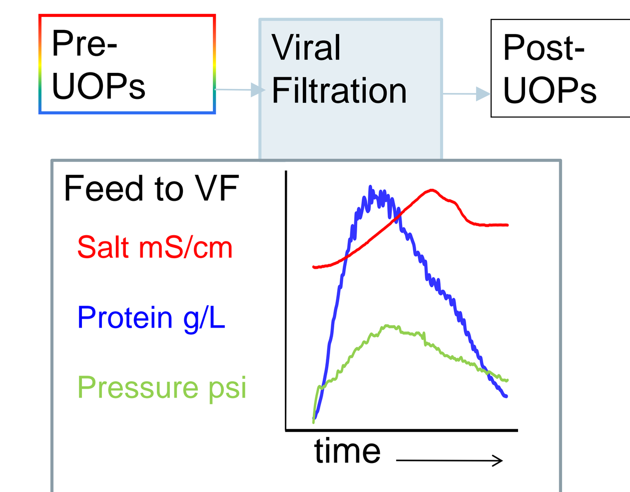
Inline spiking shows comparable LRV to standard spiking.
Inline spiking shows improved filtrate throughput

Linked processing

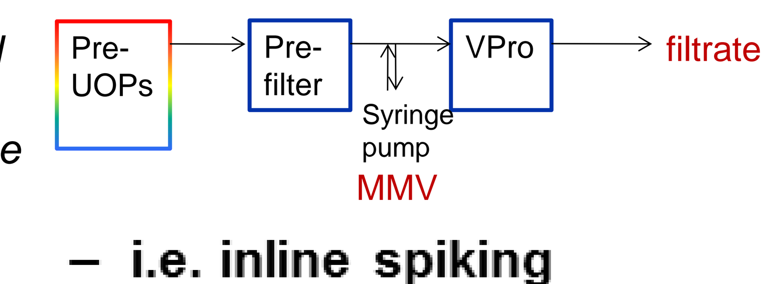
Sequential unit operations are started before the previous steps are completed.

Pre-UOPs → Viral Filtration → Post-UOPs

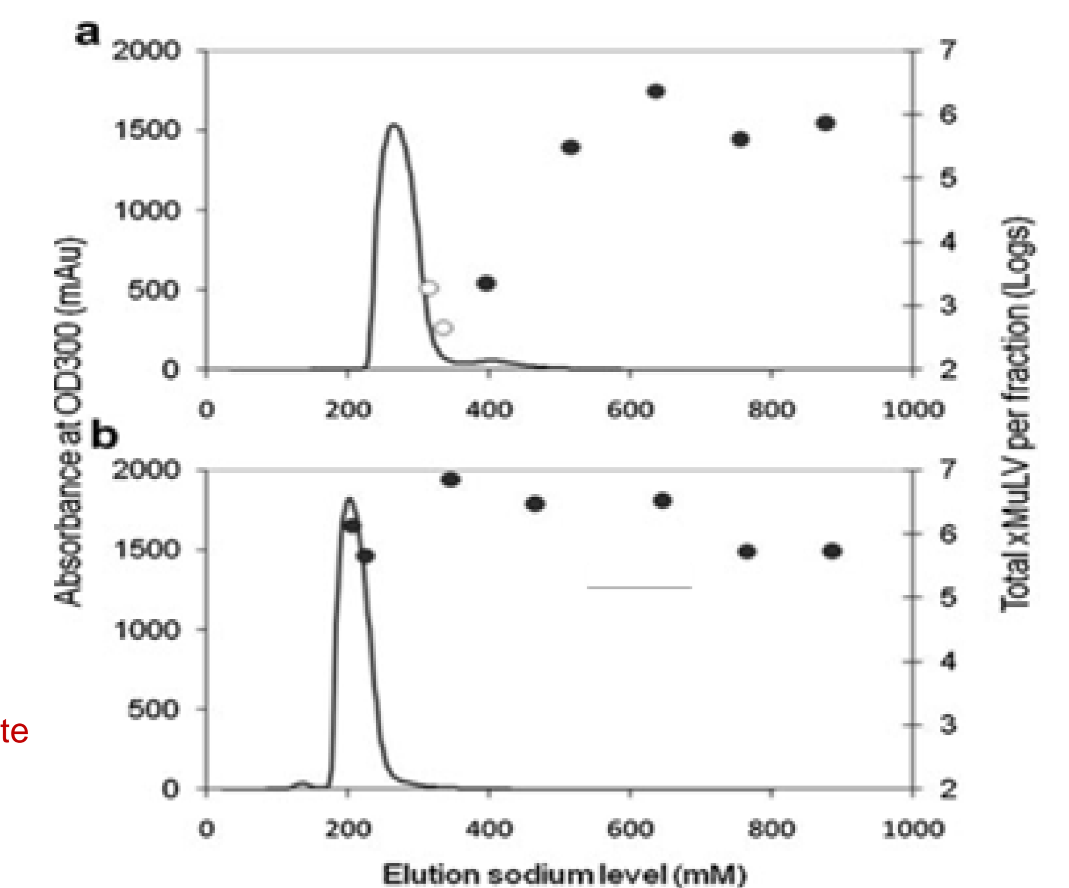
Virus filter or other clearance step sees variable operating feed parameters



ICH Q5A (1996) Measure virus removal using scaled down models that mirror large scale production to ensure that the virus removal results can be translated to the biotherapeutic product.



– i.e. inline spiking

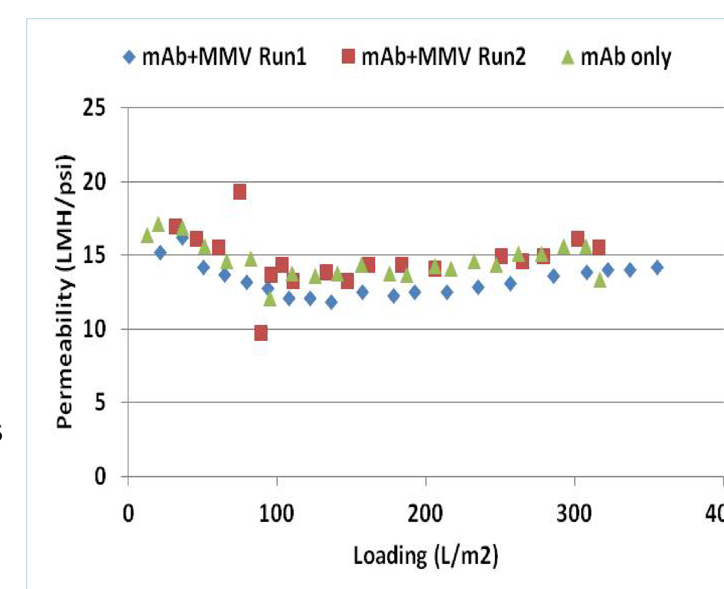


MuLV elution from upstream CEX a) pH5, b) pH6

Linked processing data

Inline spiking enables linked process validation study

>5 LRV consistent with batch processes
Feed & sample loads match (no inactivation)
Repeatable



Method allows for spiking during elution segments. Amgen CEX data indicates virus load uniform across elution to lag behind elution.

Run 1	Titer (LogTCID/ mL)	Vol (mL)	Viral Load (Log TCID)	
iSS (feed syr.)	6.68	5.1	7.86	Avg 7.85
iHC (sample syr.)	5.30	112	7.83	
Pool	-0.62	112	1.91	LRV 5.9

Run 2	Titer (LogTCID/ mL)	Vol (mL)	Viral Load (Log TCID)	
iSS (feed syr.)	6.55	3.1	7.52	Avg 7.47
iHC (sample syr.)	4.93	104	7.42	
Pool	≤-0.62	104	≤1.88	LRV ≥5.6

Data courtesy Megan McClure, Viveka Raol, Amgen

Summary

Inline spiking methodology:

- Improves filter throughputs
- More representative of manufacturing inline operation
- Demonstrates virus LRV comparable to standard spiking
- Consistent
- Relatively easy to operate
- Useful where proteins form plugging foulants
- Facilitates validation of linked processes

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