<u>Poster Presentation</u>

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Poster #1

Systematic Development of Sanitization Protocols for a Chromatographic System Designed for Biotherapeutics Purifications

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Abstract

The development of protein derived therapeutic drugs through the application of genetic engineering and biotechnolgy techniques has resulted in the design of multi-step, multi-technology processes for producing the target products. Considerable development time must be invested to optimize the fermentation, harvesting and purification steps to yield reproducible, high yield, economical products. Once developed, implementation of the procedures requires comprehensive attention to Good Manufacturing Practices (GMP), Good Laboratory Practices (GLP) and other guidelines specified by regulatory agencies. These guidelines address all aspects of the production process, including facility design, equipment selection, personnel training and process validation.

Many of the process requirements for product recovery and purification are met by combinations of tangential flow filtration and chromatography separation techniques. Consequently, increasing emphasis is being placed on the development of separation techniques that meet the specifications for final product release. Key components of the validation process for chromatographic separations are equipment design and operation certification, column packing certification and standard operating procedures (SOP).

Clean-in-place procedures (CIP) become a part of the master method and require validation as part of the entire process. Consequently, validation of CIP procedures requires definition of what "clean" is for a given process. Documentation of sanitization effectiveness, the chemical process of killing vegetative microbial cells, on microbial contaminants is also necessary for certain types of equipment. It is critical that the analytical techniques used in the validation exercise for sanitization procedures are sensitive, accurate and reproducible. This article describes the extensive microbial challenge of a Waters[™] 650 Advanced Protein Purification System and the effectiveness of both sodium hydroxide and ethanol solutions in achieving multilog reduction of microbial contamination. In addition, it describes the application of a membrane filtration method for the highly sensitive measurement of microbial contamination in chromatographic eluents. Experimental

<u>System:</u>

Waters[™] 650 Advanced Protein Purification System Waters 486 Tunable Absorbance Detector

Challenge Organisms:

Pseudomonis aeruginosa (ATCC #9027) Acholeplasma laidlawii (ATCC 23206)

Microbial Analysis Monitors:

Millipore $0.45\mu m$ or $0.22\mu m$ Mixed Esters of Cellulose Membranes, 37mm Diam.

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Key Components of the Validation Process

- Equipment Design
- Operation Certification
- Column Packing Certification
- Standard Operating Procedures

Summary of Final Product Release Testing

Tests	Biological Products Derived from					
	Monoclonal ^a Antibodies	Human Cell	Mouse/Hamster Cell	Bacteria	Yeast	
General salety	x	x	х	X	Y	
Sterility	X	x	x	x	v	
Rabbit pyrogen/LAL	x	х	x	×		
Mycoplasma	b	ь	b	NR	A NR	
Contaminating DNA	x	x	x	X	Y	
Viral contamination	b	b	<u>b</u>	NR	NR NR	

^aCurrent FDA recommendations for Phase 1 studies(6). Actual requirements should be discussed with FDA.

^bDepends on findings of unprocessed bulk.

X=required; NR=not required

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This table originally appeared in "Lot Release - Final Product Safety Testing" by L.J. Schiff, et.al., Reprinted from BioPharm 5, No. 5, 36-39 (1992) with permission of Advanstar Communications

Sanitization Protocols

- The Chemical Process of Killing Vegetative Microbial Cells
- Protocols Become Part of Master Method and Require Validation
- Require Bioburden Analysis for Validation
- The Analytical Techniques Must Be: Sensitive Accurate Reproducible Quantitative

Sources of Microbial Contamination in a Chromatography System

- Outside Environment Water Source Elution Buffer Preparation System Handling Animal Serum Nutritive Additives
- Host Cells
 Tissue and Cell Culture

Challenge Organisms

• Pseudomonas aeruginosa

More Resistant to Sanitization Than Other Gram Negative Bacteria

Originates In Water And Soil

• Acholeplasma laidlawii

Bacteria of Most Concern for Contamination of Host Cell Preparations



Protocol Development Strategy

• Extensive System Challenge

10⁷-10⁹ cfu/mL Initial Challenge All System Flow Paths Overnight Challenge To Allow For Cell Attachment

Sanitizers Evaluated

1.0N NaOH 70% Ethanol

Parameters Evaluated

Contact Time Static vs Continuous Flow 4-Day Sanitization Effectiveness



Concentration of *P.aeruginosa* as A Function of Time

Time (hr)	0.76% Saline Control	70% Ethanol	1.0 N NaOH
Pretest	9.00E+07	6.00E+07	1.03E+08
0	TNTC	NG	NG
1	TNTC	NG	NG
2	TNTC	NG	NG
3	TNTC	NG	NG
4	TNTC	NG	NG
5	TNTC	NG	NG
6	TNTC	NG	NG
24	TNTC	NG	NG

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P. aeruginosa (cfu/ml) Adsorbed to Polystyrene

Chromatography System Flow Schematic

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Quantitative Analysis of Test Effluent

- Designed So That Low Levels of Vegetative Microorganisms Can Be Detected (<10 cfu) In Large Volumes Of Fluid
- Based On Membrane Filter Method

Entire Effluent (100mL) Is Passed Through a 0.45µm or 0.22µm Mixed Esters Of Cellulose Filter

Filter Plated Onto Agar Substrate

 Allows Evaluation of 100% of Effluent and Direct Enumeration of the Microbial Colonies

P. aeruginosa Microbiological Analysis

- Initial Concentration Determined Via The Dilution And Spread Method On TSA Plates
- -100 mL Peptone Test Sample Collected On Sterile 37 mm,0.45 μm Cellulose Membrane
- Membrane Plated Upon TSA
- Incubate @ 30°± 2°C For 7 Days
- Enumerate Colonies

Cleaning Method	Initial Challenge Concentration (cfu\mL)	Day 1	Day 2	Day 3	Day 4	LRV
1N NaOH, static, 60 minutes	4.98x10 ⁷	0	TNTC	TNTC	•	NA
1N NaOH, 10 mL/min, 95 minutes	5.50x10 ⁷	0	0	TNTC	TNTC	NVA
1N NaOH, 1.5 mL/min, 60 minutes	3.98x10 ⁷	•	0	80	•	5. 69
70% ETOH, static, 60 minutes	6.75x10 ⁷	8	TNTC	TNTC	•	NVA
70% ETOH, static, 16-18 hours	7.50x10 ⁷	0	3	•	TNTC	NA
70% ETOH, static, 16-18 hours Replicate experiment #1	1.06x10 ⁸	0	0	•	0	8.02
70% ETOH, static, 16-18 hours Replicate experiment #2	8.47x10 ⁷	0	0	•	0	7.93
70% ETOH, static, 16-18 hours Replicate experiment #3	9.38x10 ⁷	0	0	•	0	7.97
70% ETOH, static, 16-18 hours Repl icate experiment #4	6.03x10 ⁷	0	0	•	0	7.78

Pseudomonas aeruginosa Bloburden Assessment

The presence of *P. aeruginosa* contained in 100 mL peptone water samples was assessed both prior to and after system challenge and cleaning was assessed by TSA plating. TNTC denotes "Too Numerous To Count".

Indicates that a sample was not taken on that day.

Non Applicable. The LRV could not be calculated because no real number exists. N/A

Scanning Electron Microscopy of Chromatography System Tubing Lumen Walls.

The effectiveness of the 70% aqueous ethanol sanitizer in destroying sessile colonies of *P. aeruginosa* is demonstrated by the micrographs of sections of system tubing removed prior to (A) and after (B) implementation of the sanitization protocol.





A. laidlawii Microbiological Analysis

- Initial Concentration Determined Via The Drop And Stab Method On FCA Plates
- 100 mL Peptone Test Sample Collected In Sterile Graduated Cylinder
- Sample Filtered Through A 47 mm, 0.22 μm Cellulose Filter Using A Vacuum Filter Funnel
- Membrane Plated Upon FCA
- Incubate @ 37°± 2°C, 7% CO₂ For 7 Days
- Visualize Colonies By Staining The Membrane Filter With Dienes Stain
- •Enumerate Colonies Using A Stereomicroscope at 40X Power

Cleaning Method	initial Challenge Concentration (cfu\mL)	Day 1	Day 2	Day 3	Day 4	LRV	<u> </u>
70% ETOH, static, 16-18 hours	3.96x10 ⁸	0	0	•	0	8.59	
95% ETOH, static, 16-18 hours	2.62x10 ⁷	0	0	•	75	5.54	
1N NaOH static, 16-18 hours	1.47x10 ⁸	0	0	•	0	8.17	
1N NaOH static, 16-18 hours Replicate experiment #1	1.1 9x10⁹	0	-	•	0	9.07	
1N NaOH, static, 16-18 hours Replicate experiment #2	1.18x10 ⁹	0	38	•	>100 <300	6.59	
1N NaOH, static, 16-18 hours Replicate experiment #3	2.60x10 ⁸	0	0	•	>100 <300	5.94	
1N NaOH, static, 16-18 hours Replicate experiment #4	3.48x10 ⁸	0	-	•	0	8.54	
1N NaOH, static, 16-18 hours Replicate experiment #5	3.85x10 ⁸	0	~	•	0	8.58	
1N NaOH, static, 16-18 hours Replicate experiment #6	3.37x10 ⁸	0	0	•	0	8.53	

Acholeplasma laidlawii Bloburden Assessment

The presence of A. laidlawii contained in 100 mL peptone water samples was assessed both prior to and after system challenge and cleaning was assessed by FCA plating. Indicates that a sample was not taken on that day. Indicates that Staphylococcus contamination was detected pre-sanitization but not post

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~ sanitization.



- Effective System Sanitization May Be Accomplished Using NaOH and Ethanol Solutions
- LRV = 8-9 Is Documented
- Sanitizer And Contact Time Are Critical Parameters
- Membrane Filter Method Provides The High Sensitivity Required For Low Level Bioburden Analyses
- Protocols Must Be Validated For Bioburden Anticipated in a Given Process
- Sanitization Effectiveness Should Be Monitored Several Days Following Protocol Implemetation