Development of a cost effective and scalable purification process for Vi polysaccharide.

Sudeep Kothari, PhD
Former Senior Research Scientist
September 26, 2017

International Vaccine Institute

Moral obligation

Every child should have the opportunity to receive high quality, safe and efficacious vaccines to protect them from life threatening infectious diseases

Limited funding

Funding for vaccination is limited so in order to get the broadest coverage in resource poor countries

vaccines must be affordable

How does VPD address affordability?

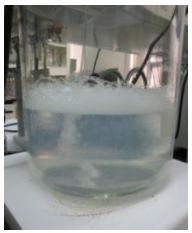
Process development:

High yielding antigen production

High recovery purification process using cost effective technology

Scalable process compatible with cGMP production






Technology Transfer:

High standards for quality, strict adherence to cGMP

Manufacturer(s) committed to low cost production and low profit margins

–Developing Country Vaccine Manufacturers

Manufacturer with capacity to WHO prequalify

Process development

Proof of principle

Process development

Process optimization
Formulation

In process control
Lot release assays

Discovery

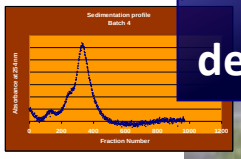
Process Scale-up

GMP production at pilot scale

Pilot scale production

product

Commercial production



Assay development

Optimization at larger scale
Understanding of process
Fine tune formulation

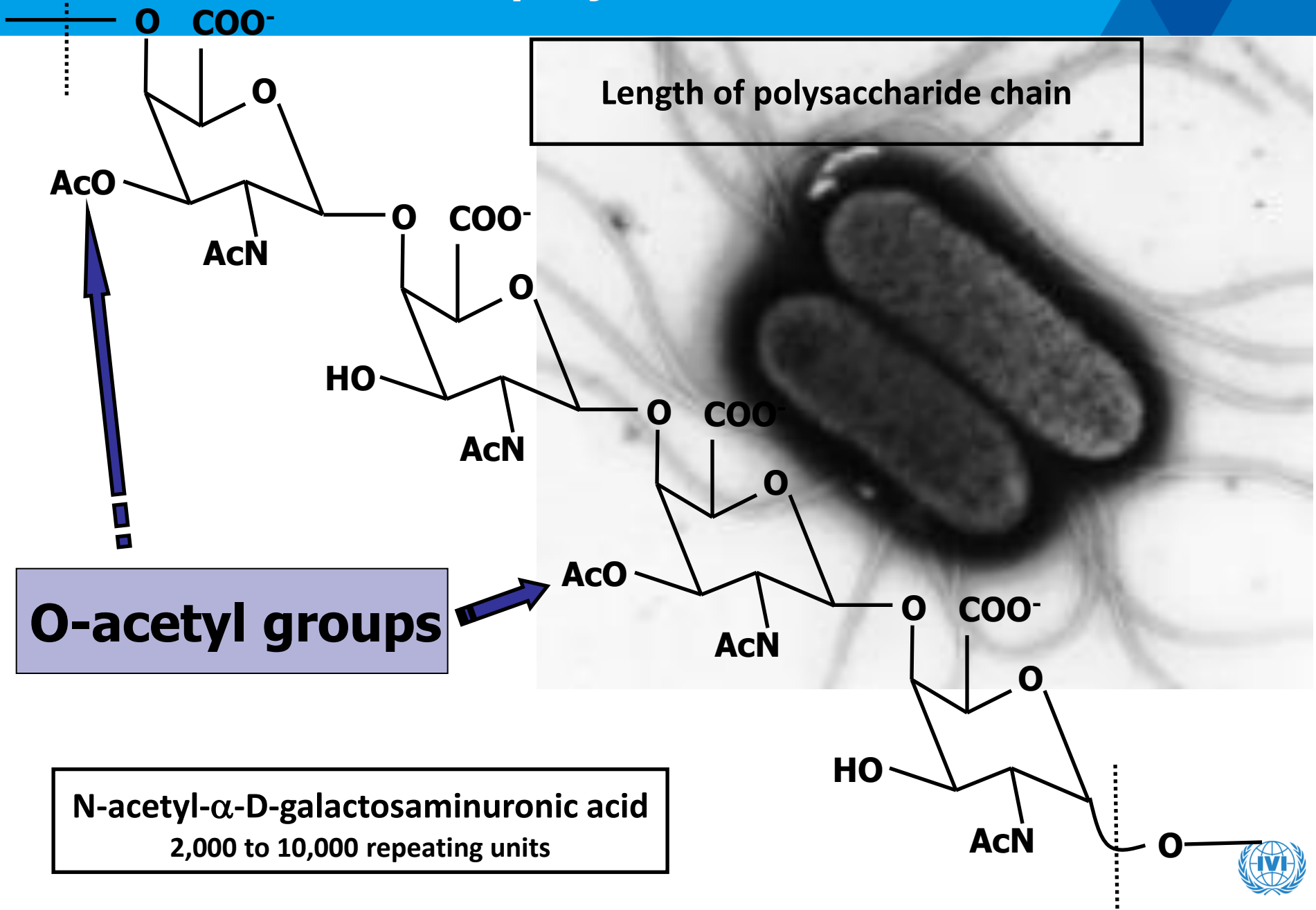
Clinical development

Discovery	PreClinical	Phase I Safety	Phase II Safety and Immunogenicity	Phase III Efficacy	Commercial production

Development of a Typhoid Conjugate Vaccine at IVI

Production and purification of Vi Polysaccharide

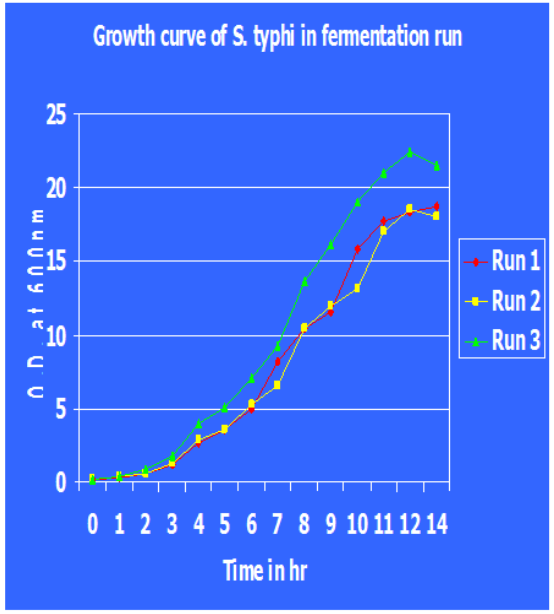
Vi polysaccharide

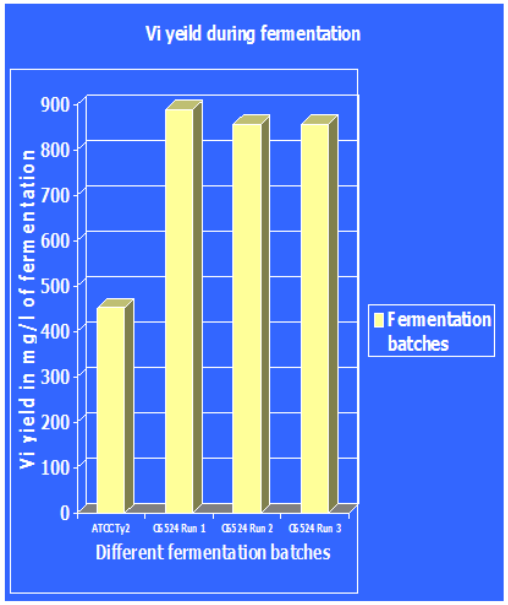


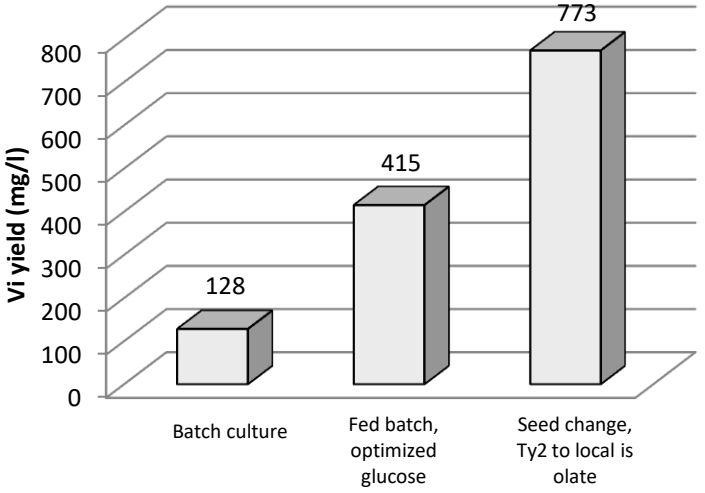
Vi production optimization

3 factors are important in Vi production

- High density bacterial culture
  - Fed batch culture increased OD_{600} four fold
- Optimal environment for expressing genes coding for Vi
  - High osmolality inhibited Vi gene expression
- Optimal chemical environment for biosynthesis and polymerization of the Vi
  - High concentrations of glucose and high pH inhibited Vi biosynthesis







Vaccine 31 (2013) 4714–4719

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A novel method for purification of Vi capsular polysaccharide produced by *Salmonella enterica* subspecies *enterica* serovar Typhi

Sudeep Kothari[a,c], Neha Kothari[a], Jeong-Ah Kim[a], Eugene Lee[a], Yeon Kyung Yoon[a], So Jung An[a], Christopher Jones[b], Woo Seok Choe[c], Rodney Carbis[a,*]

[a] Vaccine Development Section, International Vaccine Institute, SNU Research Park, San 4-8, Nakseongdae-dong, Gwanak-gu 151-919, Seoul, Republic of Korea
[b] National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK
[c] School of Chemical Engineering, Sungkyungkwan University, Suwon 440-746, Republic of Korea

Purification optimization

Downstream processing (purification of Vi)

Removal of impurities

Maximize recovery of Vi polysaccharide

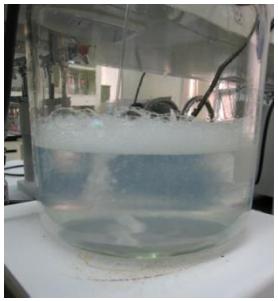
Clarification

Cells

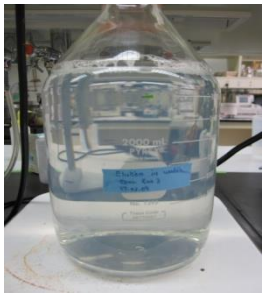
Crude Vi

Crude Vi concentrate

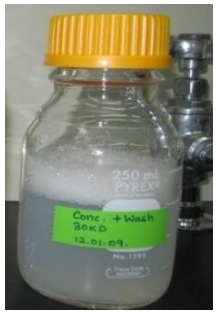
Cetavlon precipitated Vi



Ethanol precipitated Vi



Vi dissolved in water



Purified bulk Vi

Seed bank
Local Indian Isolate

Fermentation
Inactivation

Clarification of Vi using TFF

Concentration
Diafiltration

Cetavlon precipitation

Dissolve in 60% ethanol

Precipitate and wash
with 75% ethanol

Dissolve in water

$(NH_4)_2SO_4$
Precipitate impurities

Concentration / Diafiltration

Sterile filtration

Consistency lots of Vi

Batch number	Protein %	Nucleic acid %	O-acetyl content >mmol/g	Vi ELISA mg/ml	Endotoxin EU/µg
WHO Specification	**<1**	**<2**	**2.0**	**NA**	**25**
Consistency Run 1	0.4	0.3	2.3	2.6	2
Consistency Run 2	0	0.29	2.1	2.4	3
Consistency Run 3	0	0.68	2.6	1.9	4

WHO Size specifications : At least 50% of the Vi shall elute before a K_D of 0.25 (Sepharose CL4B)

All three batches comply with WHO specifications

Purification (magnitude of challenge)

In 100 litre fermentation broth

End of fermentation (upstream)

Nucleic acid:	**240 g**
Protein:	**880 g**
Endotoxin:	**100 g LPS**
Vi polysaccharide:	**70 g**

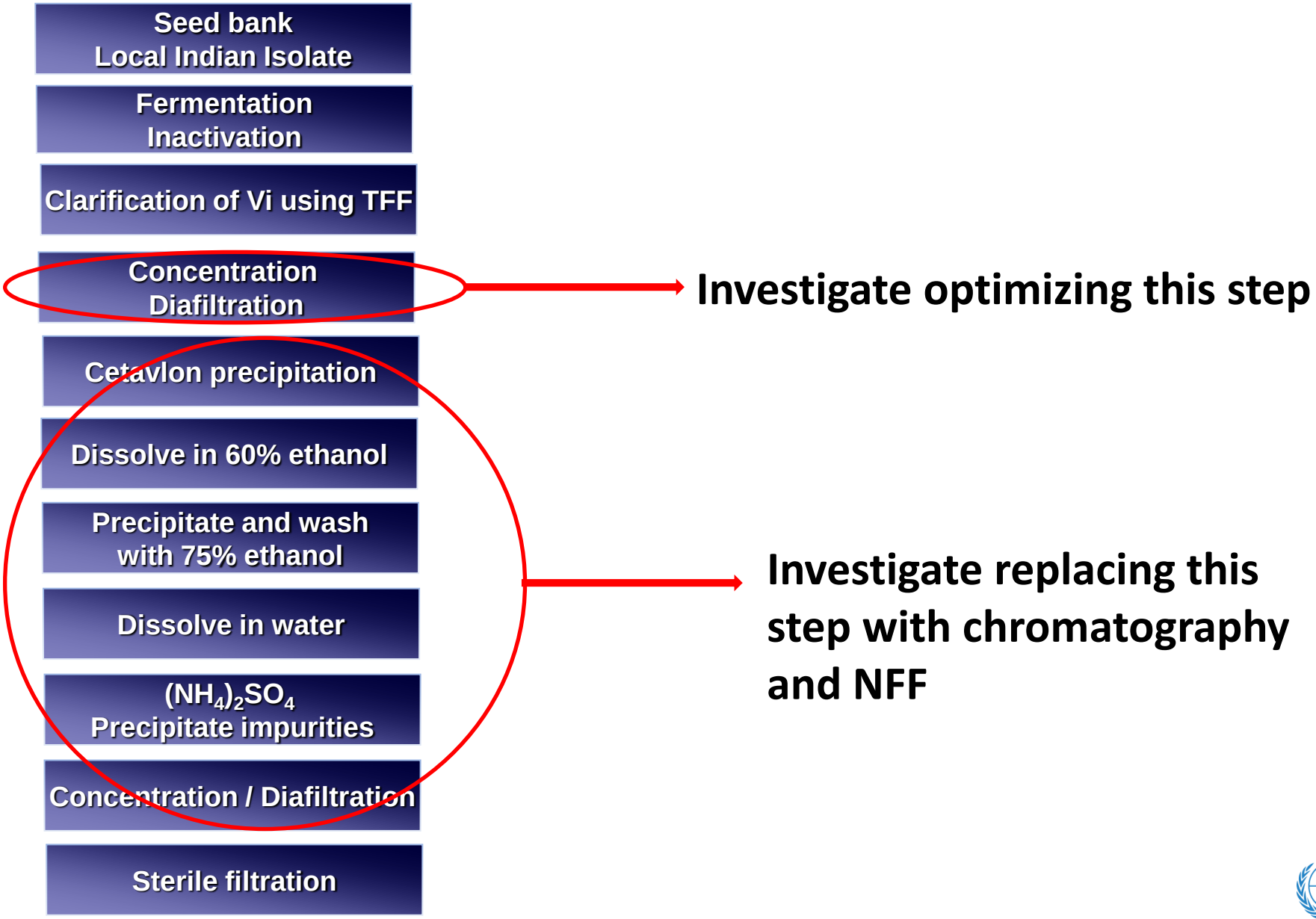
End of purification (downstream)

Nucleic acid:	**0.2 g**
Protein:	**undetectable**
Endotoxin:	**0.008 g LPS (<100 E.U./dose)**
Vi polysaccharide:	**30 g (1 million doses)**

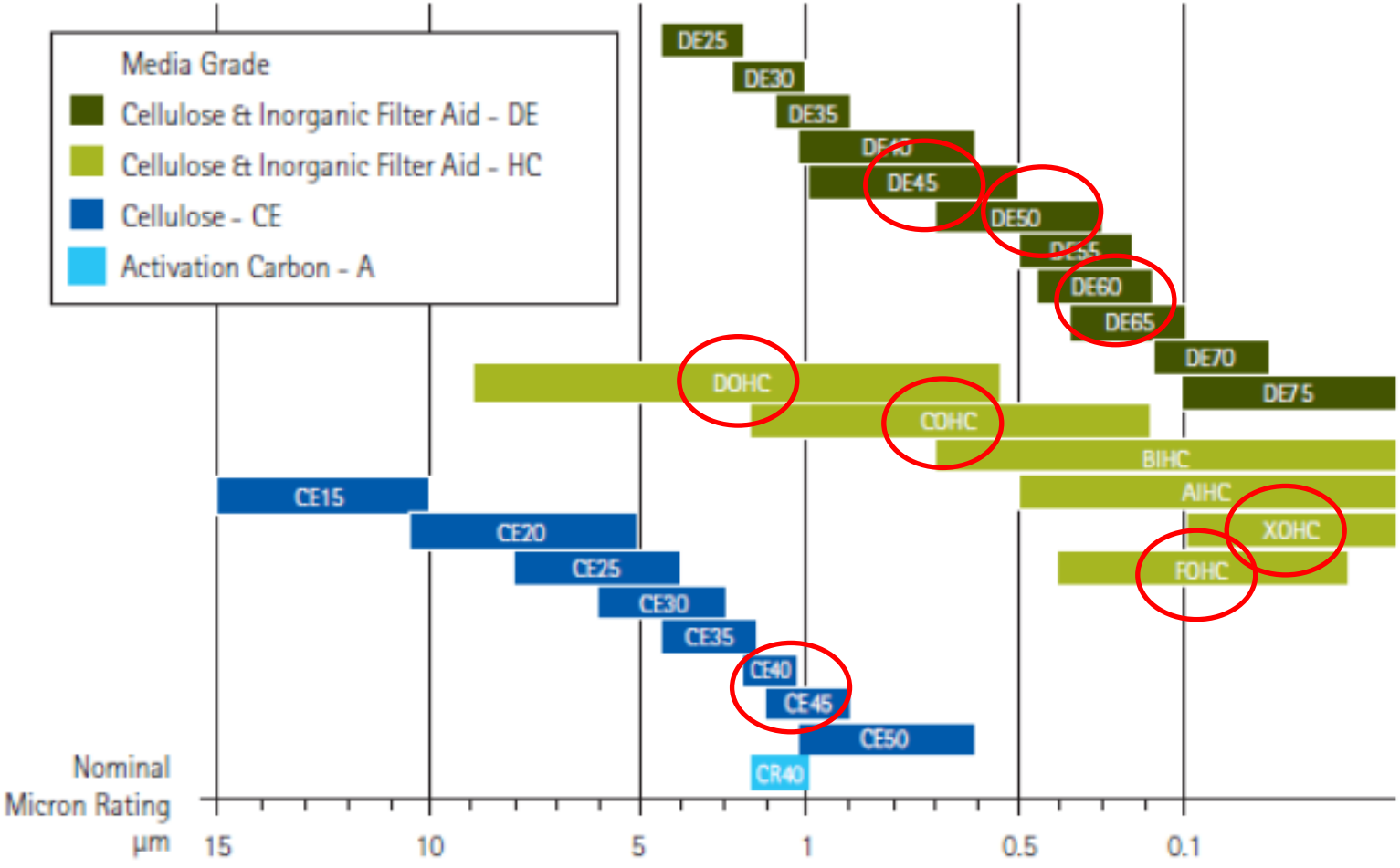
Motivation for alternative downstream process development

- **Improve Vi recovery**
- **Removal of detergent and ethanol based precipitation steps**
- **Minimizing number of purification steps**
- **Reducing production timeline and cost**
- **Ease of scalability**

Alternative Purification optimization

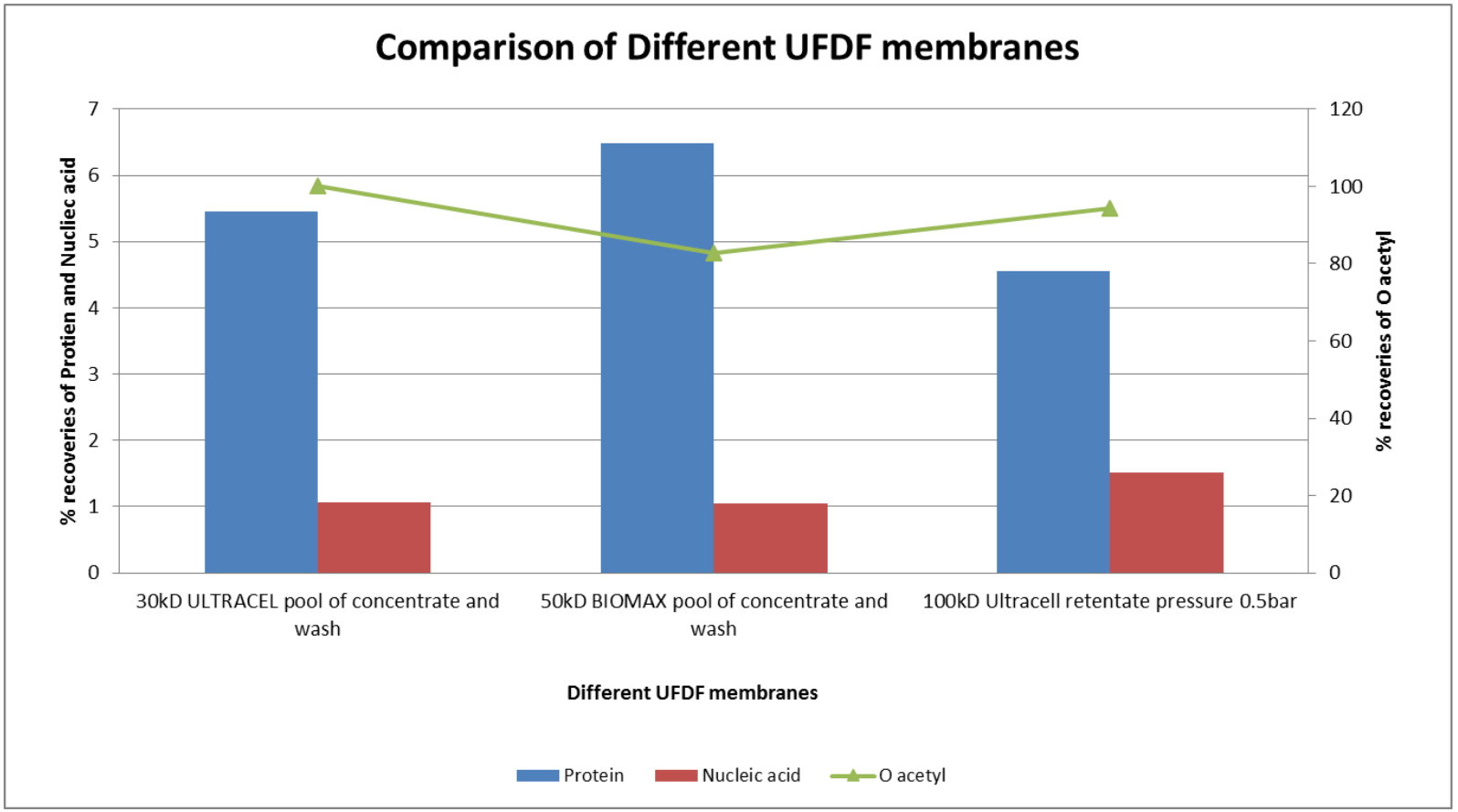


Clarification



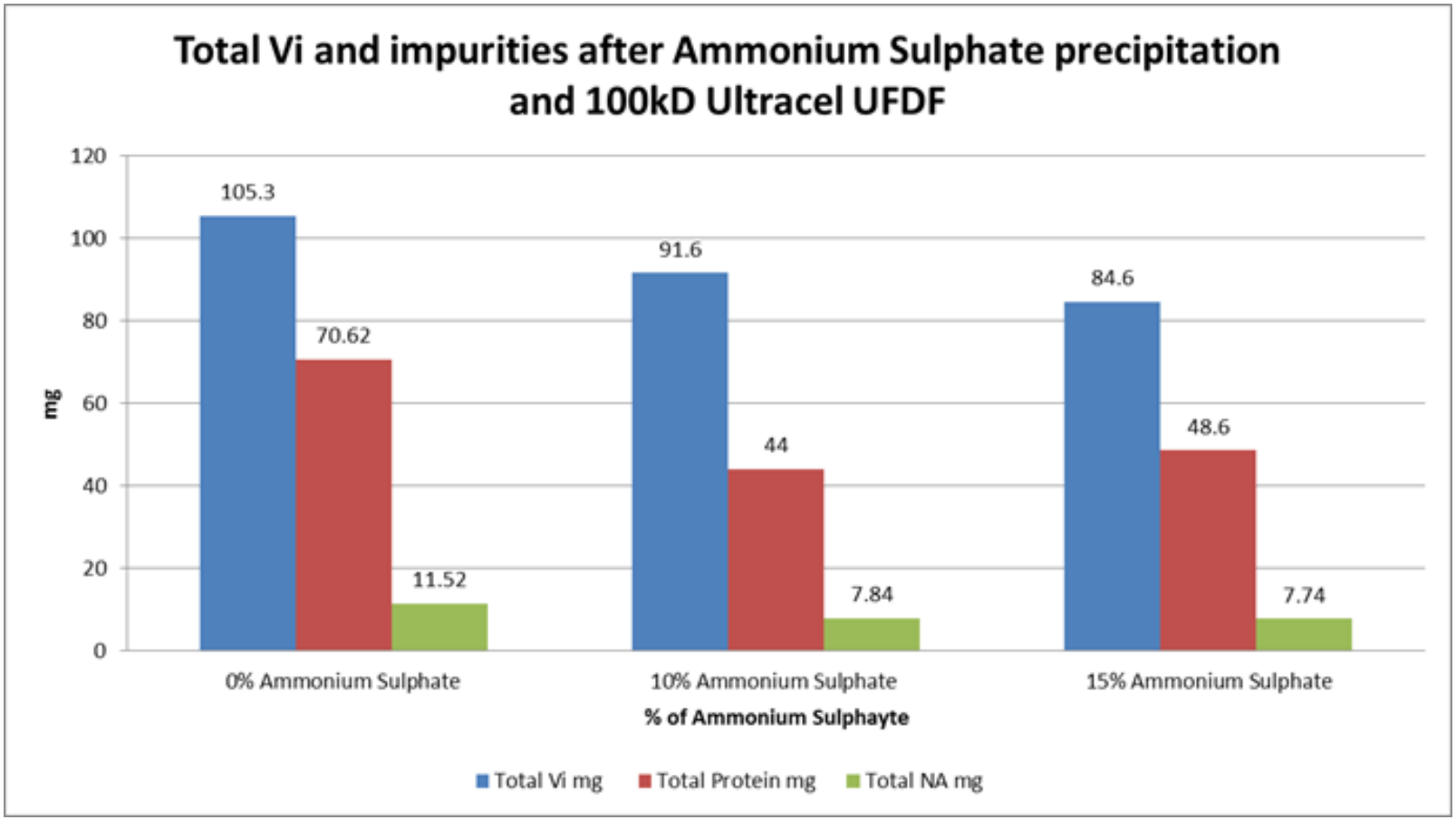
- Could not achieve clarification using depth filtration with a series of membranes at equivalent bacterial load
- Continued using 0.45µm TFF filtration

Comparison of different 88 cm² pellicon® 3 UFDF Cassettes



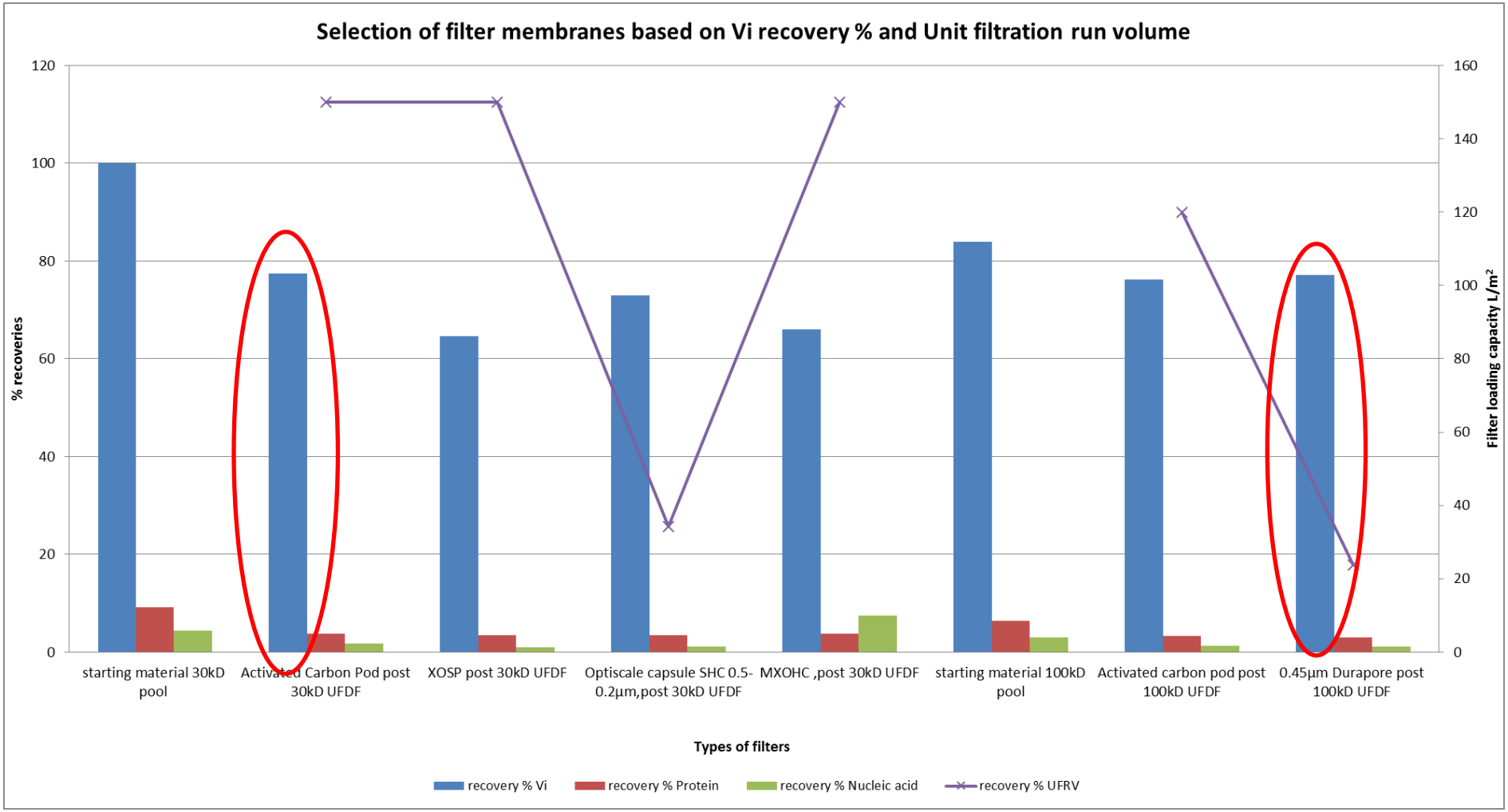
- UFDF studies with 100kD Ultracel reduced 96% of the protein and 98% of Nucleic acid contamination from the pool of 0.45µm clarified pooled samples.
- 100kD ultracel UFDF conditions are optimized with 2stage DF with 1M NaCl and Citrate phosphate buffer 6DV's each
- The Vi recoveries are close to 100%

Ammonium sulphate precipitation and concentration and diafiltration at 100kD



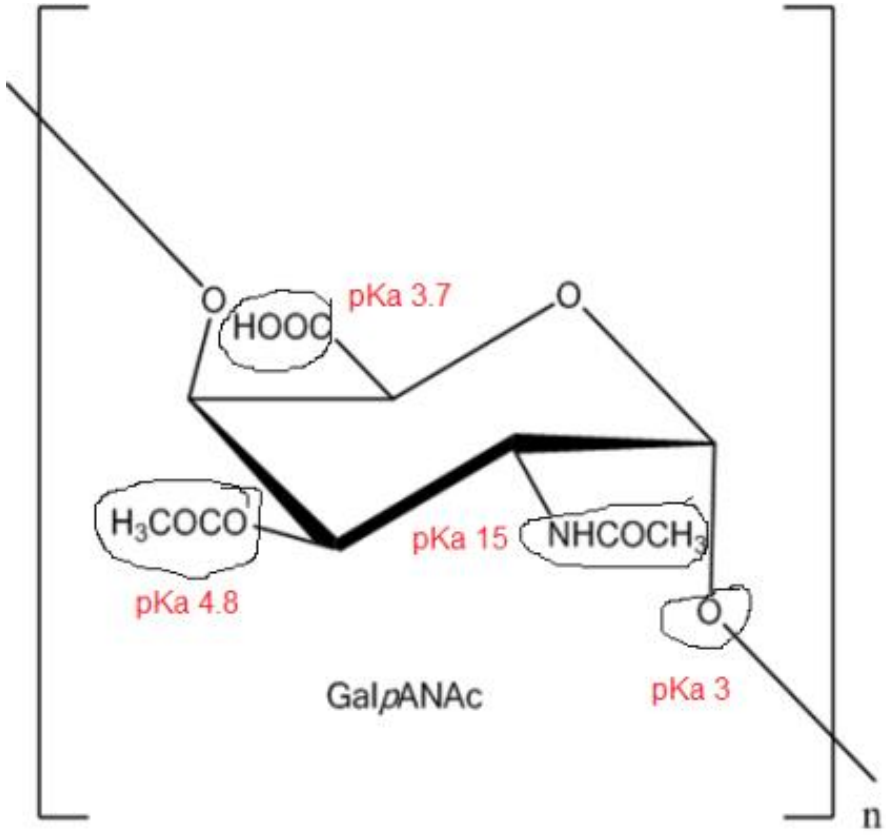
- With 10% Ammonium sulphate there is an additional 2% reduction in protein impurities and additional 0.5% reduction in Nucleic acid impurities
- Per Vi recovery at 100kD Ultracel without ammonium sulphate precipitation has only 0.01-0.02% higher impurities

Evaluation of different filter membranes pre-chromatography



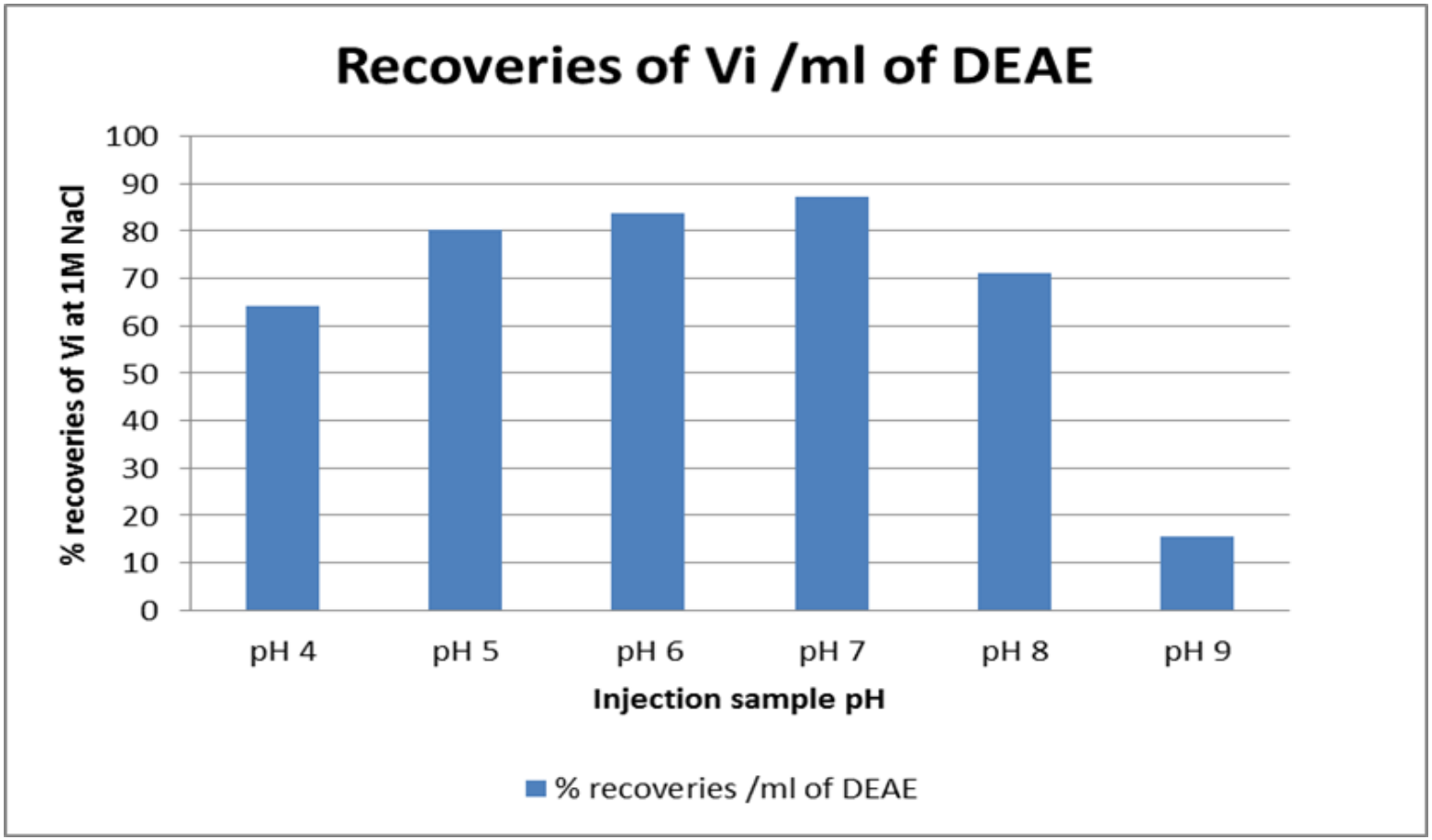
Activated carbon pod or 0.45µm Durapore can be used for filtration pre loading in Chromatography column.

Isoelectric point of Vi polysaccharide



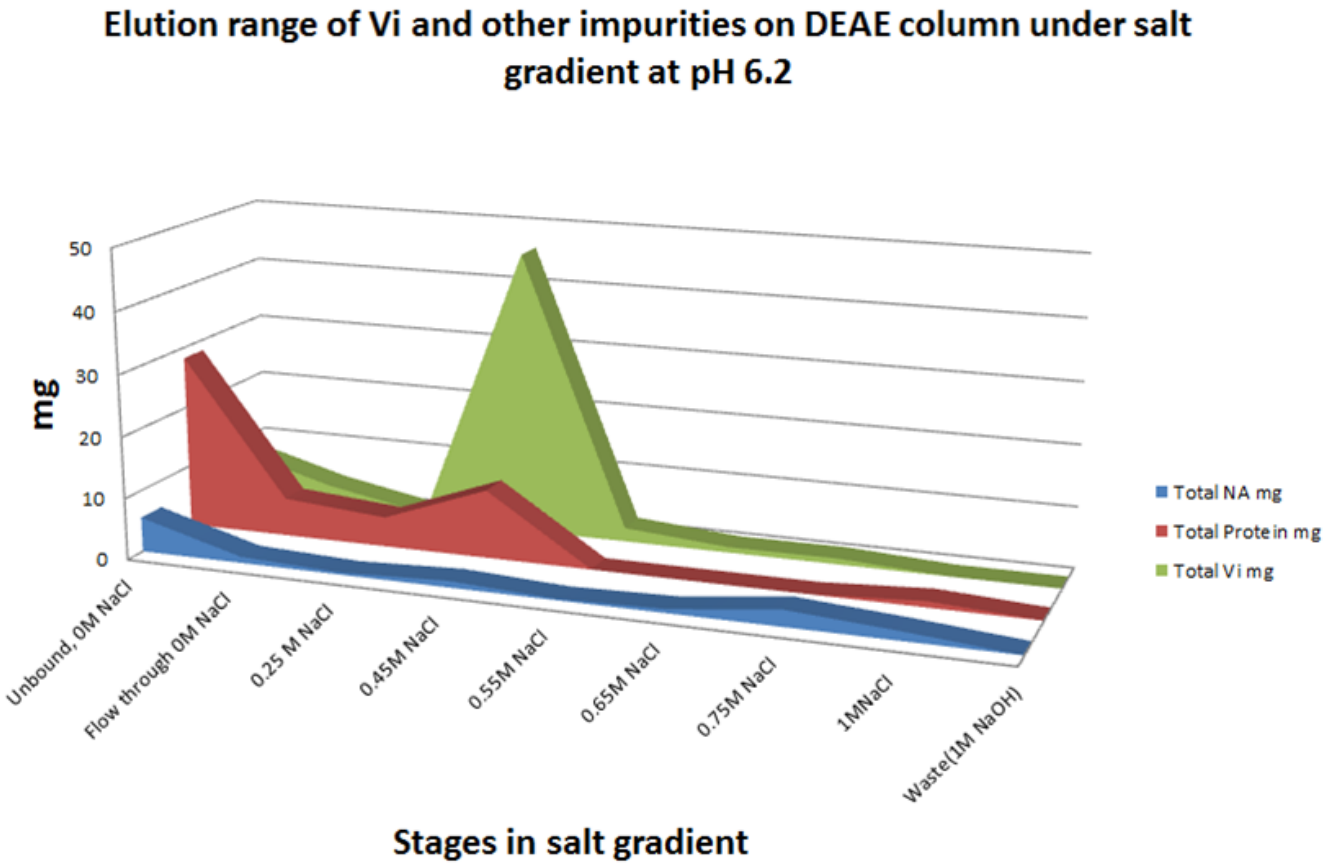
- Vi polysaccharide (N-acetyl-a-D-galactosaminouronic acid)
- 2,000 to 10,000 repeating units
- The theoretical pI of the Vi polysaccharide was determined to be around 4-4.2

Binding capacities of Vi per ml of DEAE resin at different pH conditions



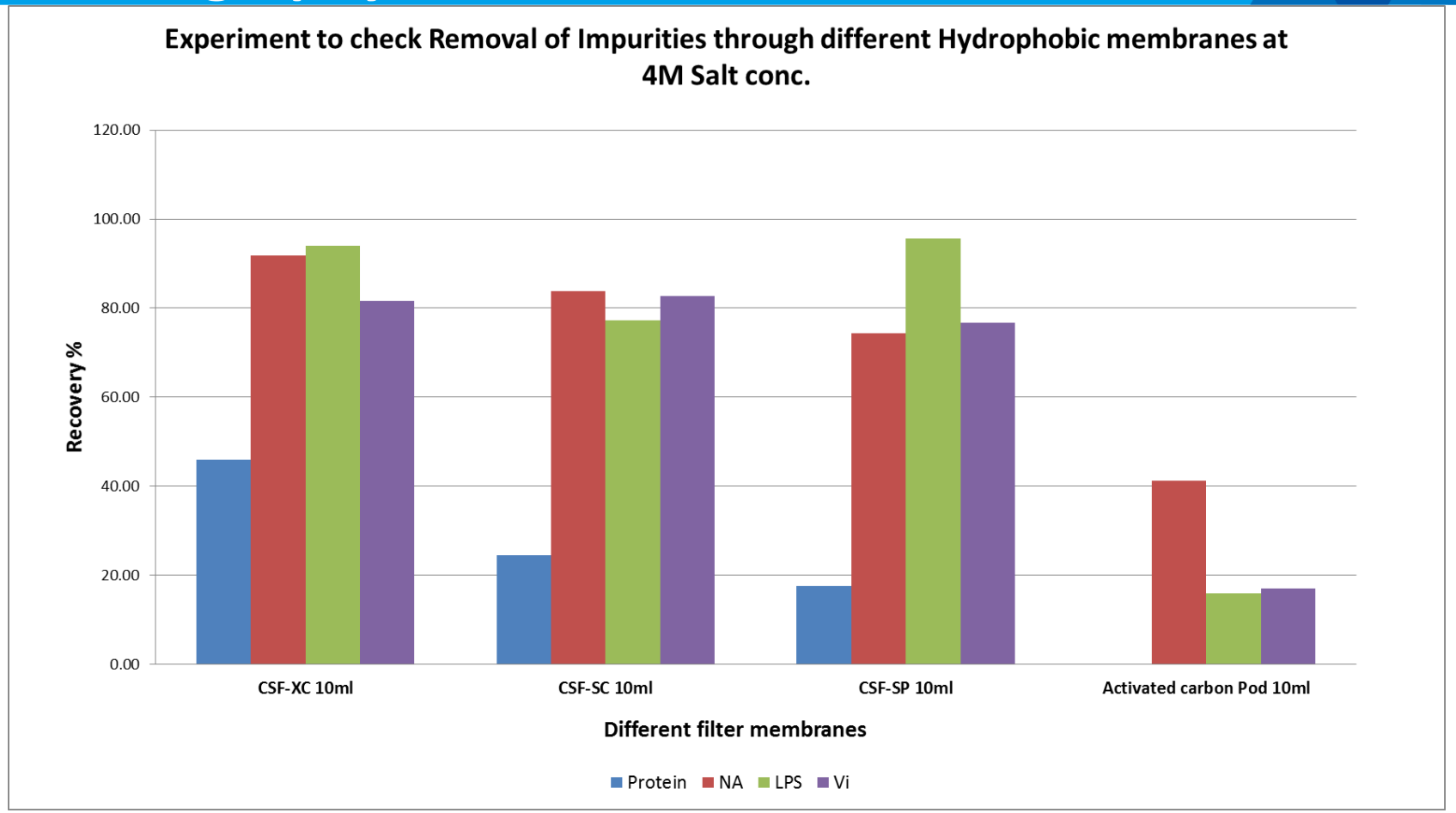
- AEX is evaluated with Weak AE (DEAE) and Strong AE resin (TMAE).
- The binding capacities of Vi is higher in Citrate phosphate buffer in comparison to MES and PBS buffer under similar pH conditions. Most optimal pH conditions are pH 6.2

Elution of Vi from DEAE column



- Vi elution is in a range above 0.25M salt concentration up to 0.55M Salt
- Binding of Vi in Citrate phosphate buffer per ml of DEAE resin is 5.6mg
- Vi yield in elution pool is 78%

Evaluation of different filter membranes post-chromatography



- Different Hydrophobic, charged, and uncharged filtration membranes were tested for the removal of endotoxin
- Activated carbon pod is able to remove 70-75% of the remaining endotoxin in the pool at 4M salt concentration but at the same time it is also binding ≈80% of Vi polysaccharide

WHO specification for Vi polysaccharide

Process type	Protein %	Nucleic acid %	O-acetyl content >mmole/g	Endotoxin EU/µg
WHO Specification	<1	<2	2.0	25
Vi purification (IVI)	0.4	0.3	2.3	2
DEAE 0.55 pool	15.7	2.3	2	8535.9

- % recovery of Vi has improved with the new process from 40-50% to 77.5%
- Alternate process still have high protein and endotoxin impurities

Conclusion and advantages of new process

- **New Vi purification process is developed without use of any detergents, ethanol precipitation and enzymatic hydrolysis.**
- **The process is close to the criteria where regulatory authorities foresee the future of Polysaccharide vaccine purification.**
- **Further work needs to be done to reduce the impurity levels within the WHO specifications for Vi polysaccharide.**
- **% recovery of Vi polysaccharide is 30-40% higher with new process.**
- **Future work will focus on additional reduction of impurities specifically endotoxin.**

Acknowledgement

International Vaccine Institute:
Wook Jin Park,
Yeon-Kyung Yoon
Dr. Neha Kothari
Dr. Viliam Pavliak
Dr. Jerome Kim

Merck-Millipore :
Elizabeth Goodrich
Charles Park
Elina Gousseinov
Daniel Koo
Mi-Ri Jung
Dr. Priyabrata Patnaik
Dr. Alex Xenopoulos
Dr. Damon Asher
Dr. Miles Shi

Thank you

Vaccines don’t save lives ,vaccination does