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Vaccine Technology IV, May 24th 2012 , Albufeira, Portugal

Purification of Cell-Based Influenza H5N1 Viruses by Liquid Chromatography Technologies

Alan Yung-Chih Hu, *Ph.D.*

Assistant Investigator

National Institute of Infectious Diseases and Vaccinology (NIIDV), NHRI

National Health Research Institutes (NHRI)

Since 1996



Moved to this campus in 2004



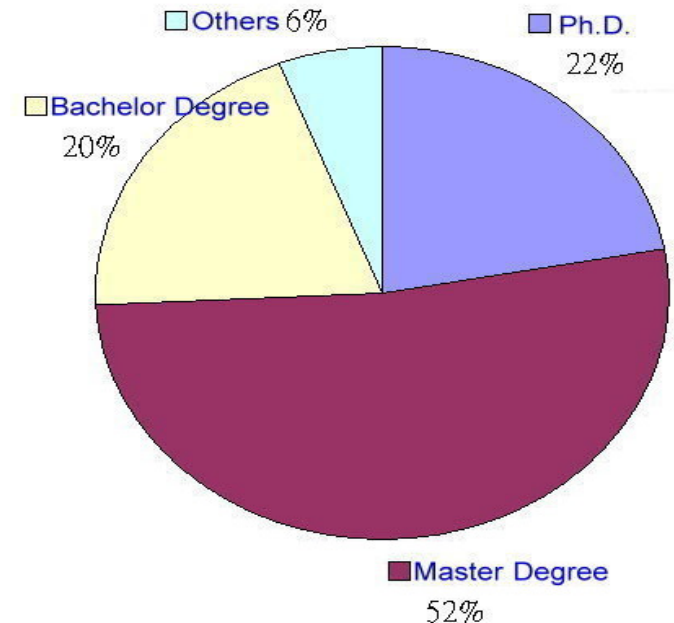
Research Programs

- National Institute of Cancer Research
- Institute of Cellular and System Medicine
 - ◆ Division of Aging-Related Musculoskeletal Diseases
 - ◆ Division of Cardiovascular and Metabolism Medicine
 - ◆ Division of Regenerative Medicine
- Institute of Population Sciences
 - ◆ Center for Health Policy Research & Development
 - ◆ Division of Biostatistics and Bioinformatics
 - ◆ Division of Gerontology Research
 - ◆ Division of Mental Health & Substance Abuse Research
- Institute of Biotechnology & Pharmaceutical Research
- National Institute of Infectious Diseases and Vaccinology
 - Division of Environmental Health & Occupational Medicine
 - Division of Medical Engineering Research
 - Division of Molecular & Genomic Medicine
 - Center for Nanomedicine Research



Institution Objectives and manpower

- Improve the health and well-being of Taiwanese
- Enhance the quality of biomedical research and medical care
- Develop medical and pharmaceutical technology
- Train and cultivate biomedical researchers
- Promote health policy research and implementation



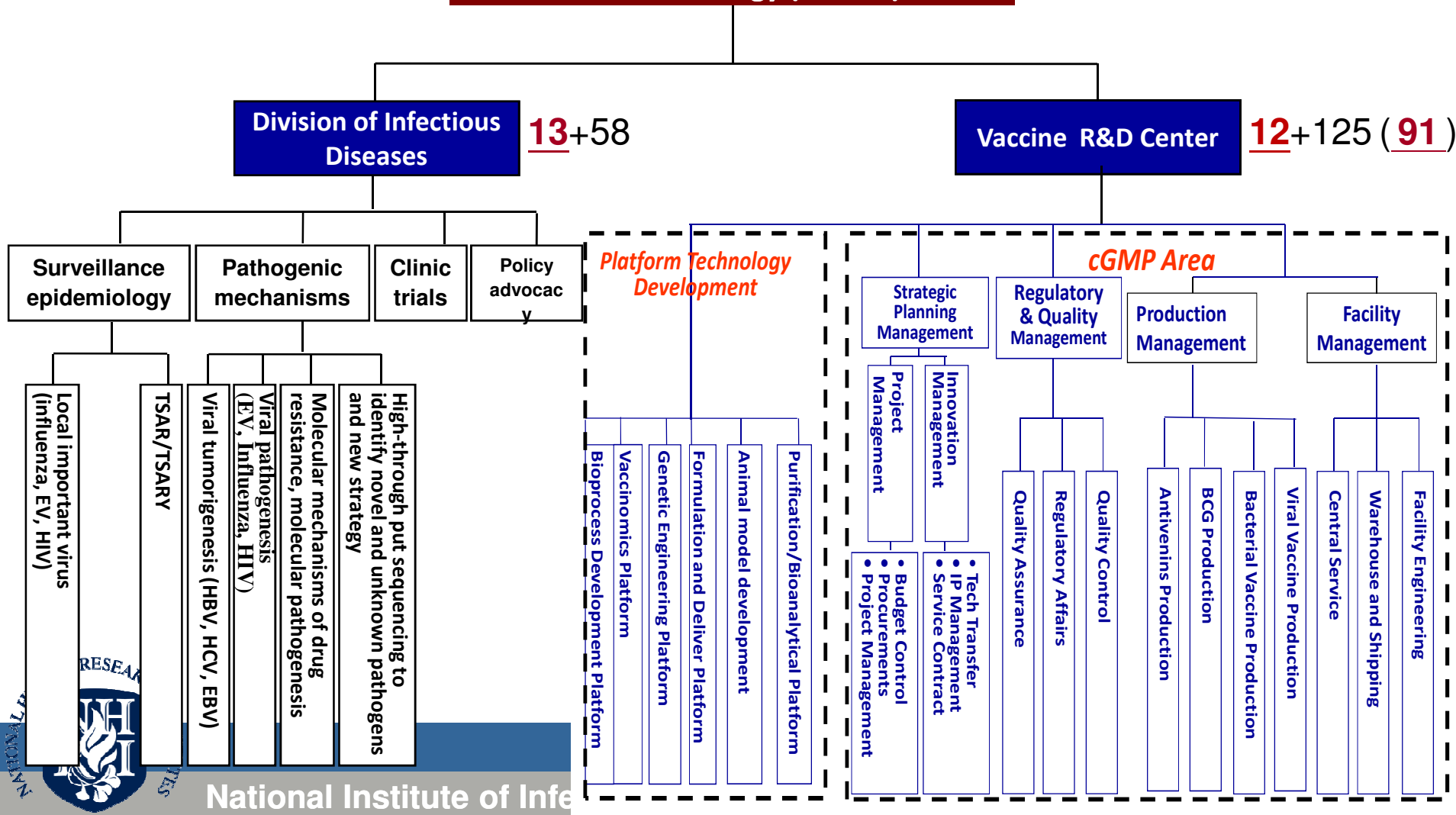
- Total: over **1600**
- As of May 2012



NIIDV Organization & Structure

Effective, May, 2011

National Institute of Infectious Diseases and Vaccinology (NIIDV)



NIIDV programs

- Antibiotic resistance, microbial genomics and infection control Advocacy
- Tuberculosis research and novel BCG/ TB vaccine development
- Emerging virus infections and vaccine development
- Tumor viruses and therapeutic development
- Novel vaccine technology and bioproduct development

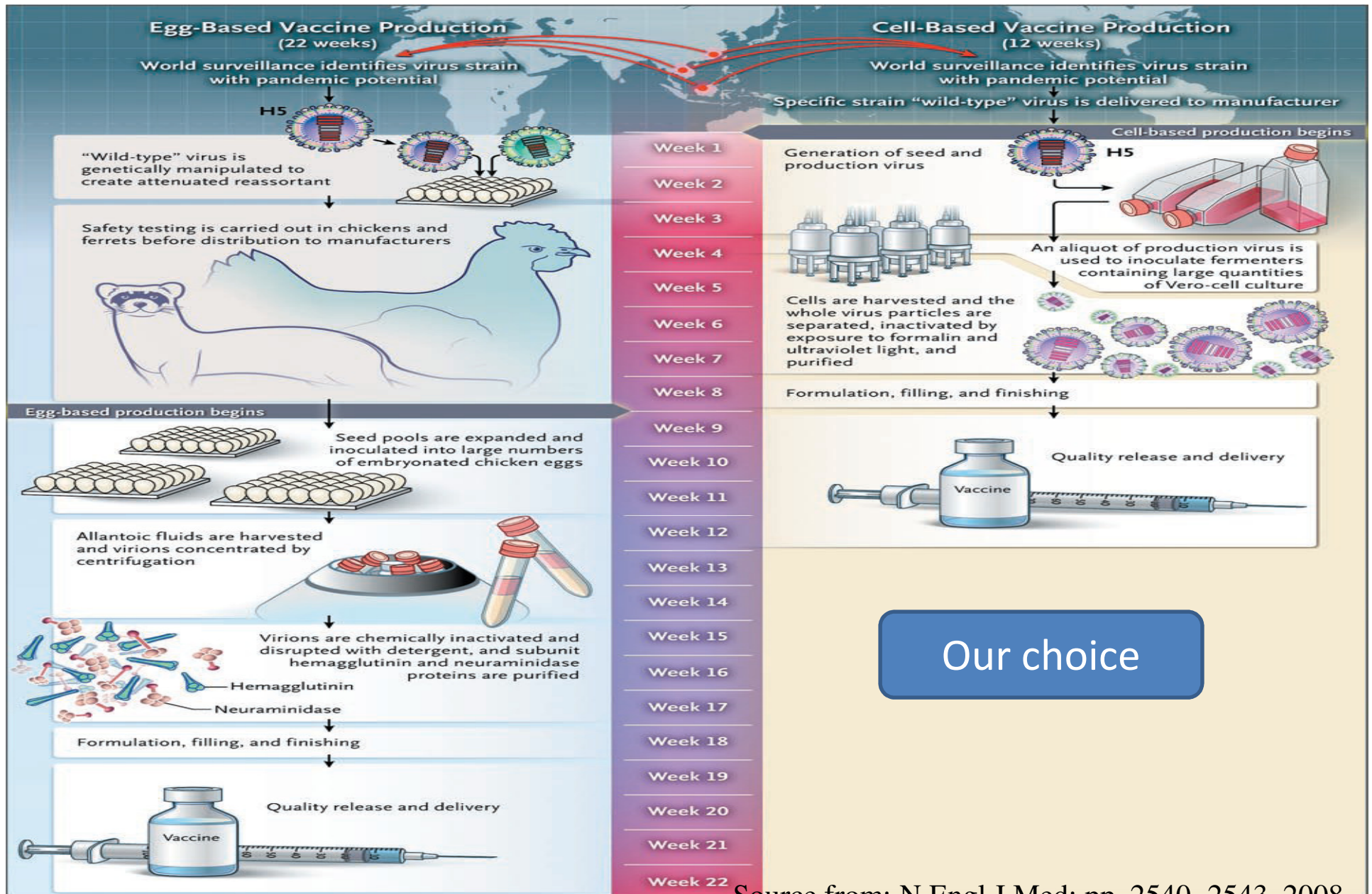


Pipelines of NHRI Vaccine Center

	Target	Status	2012 Funding
Category 1: Production contracts from Taiwan CDC	BCG Vaccine	Taking over Taiwan CDC orphan vaccine production	DOH/CDC
	Antivenin downstream production		
Category 2: Emergency manufacture	Flu Vaccine	Enhance national Bio-security and capability to produce vaccine under emergency and /or pandemic disease	DOH Regular Funding/ Industry
	Enterovirus : EV71 (B4/C4)		
Category 3: Developing vaccine with commercial value	Meningococcal Group B Vaccine	<ul style="list-style-type: none"> ● Complete product development up to phase 2 clinical trial and then technology and product transfer ● Meningococcal group B using lipoprotein technology is going to enter into Phase I in Q4/2011 	MOEA/ Industry/ DOH
	HPV Therapeutic Vaccine		
	Dengue Vaccine		
	Novel Adjuvant Development		
	RSV/PIV3 Combined Vaccine		
Category 4: Novel vaccine and technology development	Recombinant BCG/TB Vaccine	Vaccine platform technology development and novel vaccine R&D to assist, reduce the cost and timelines and enhance the product pipelines in Taiwan vaccine industry	Basic R&D by NSC/NHRI/PP project
	Universal pneumococcal Vaccine		
	Bioprocess Development		

Vaccine R&D : NHRI cGMP Pilot Plant

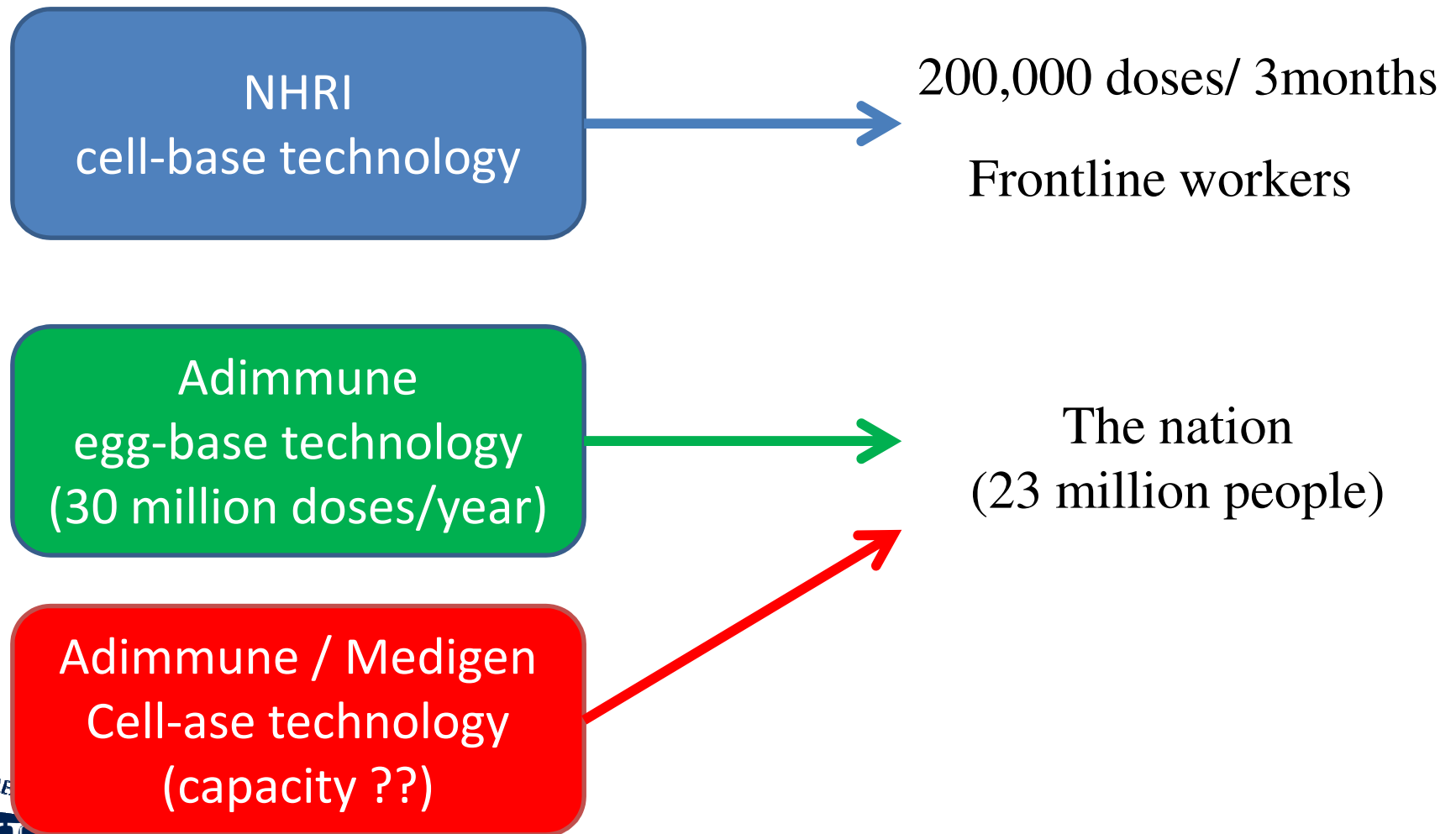




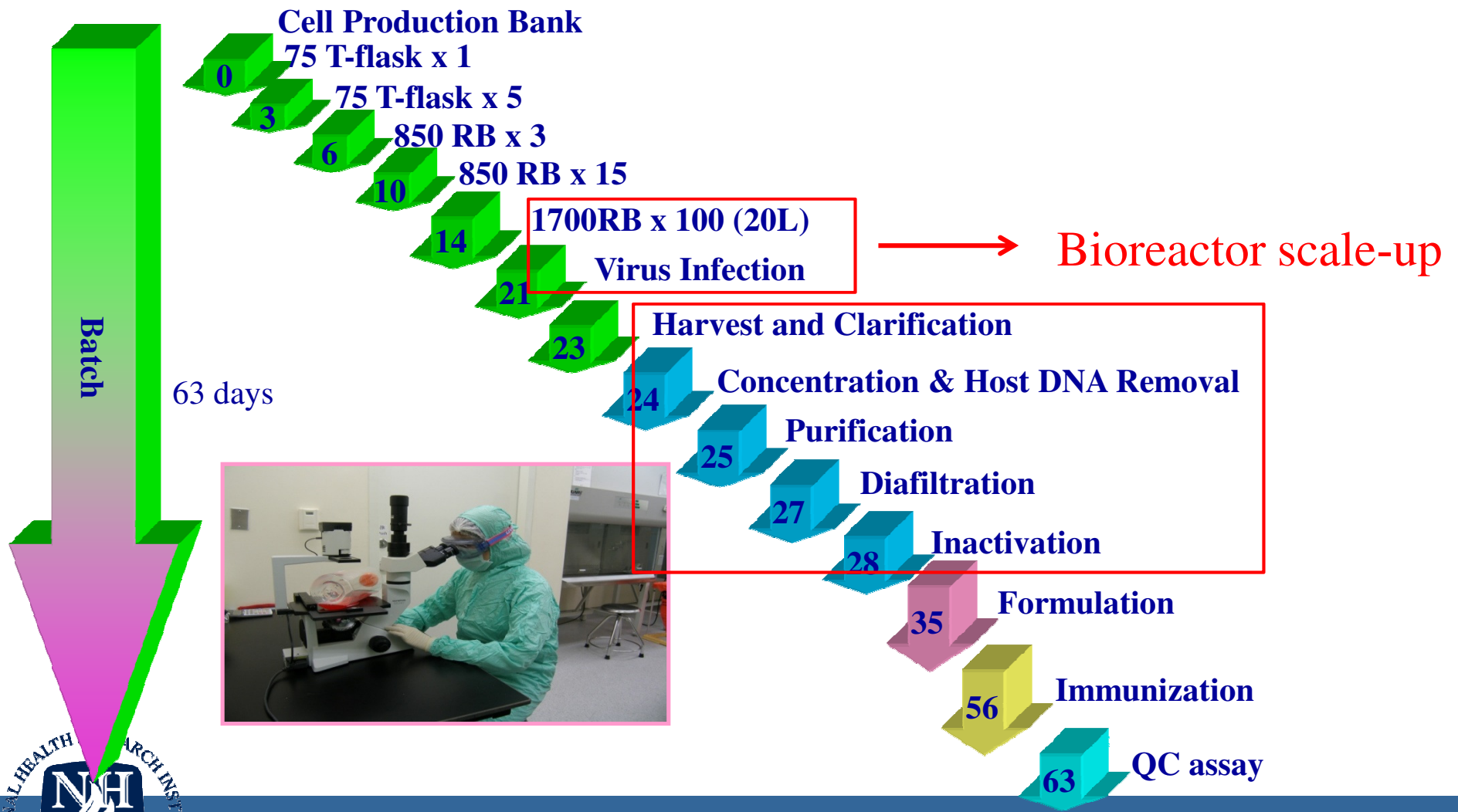
Source from: N Engl J Med; pp. 2540~2543, 2008



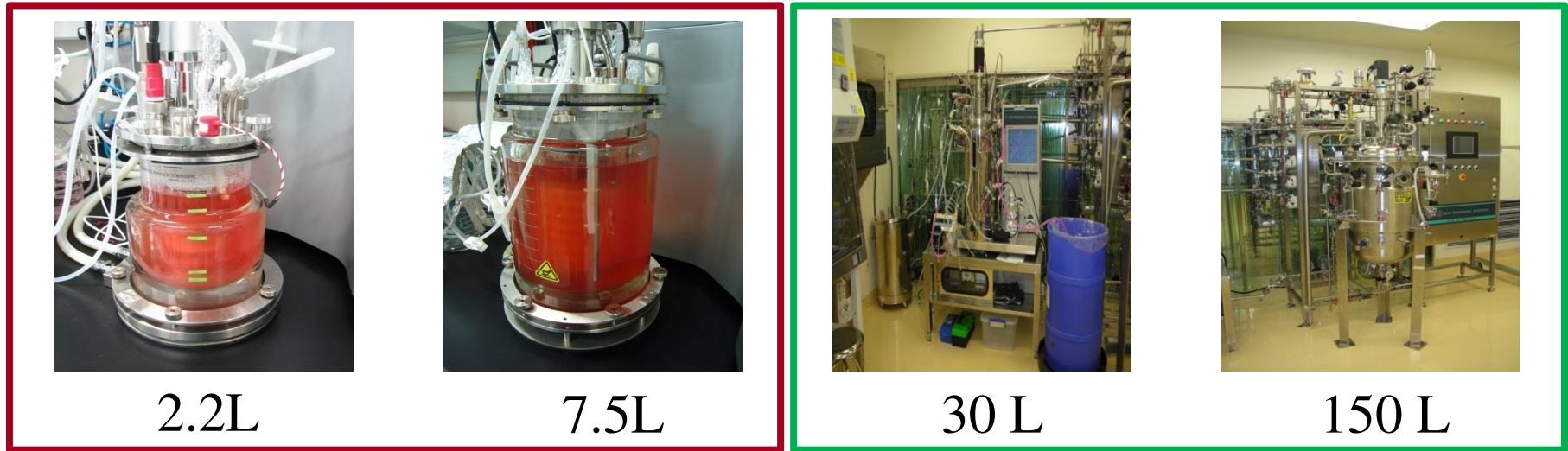
Pandemic manufacturing strategy for H5N1 preparedness



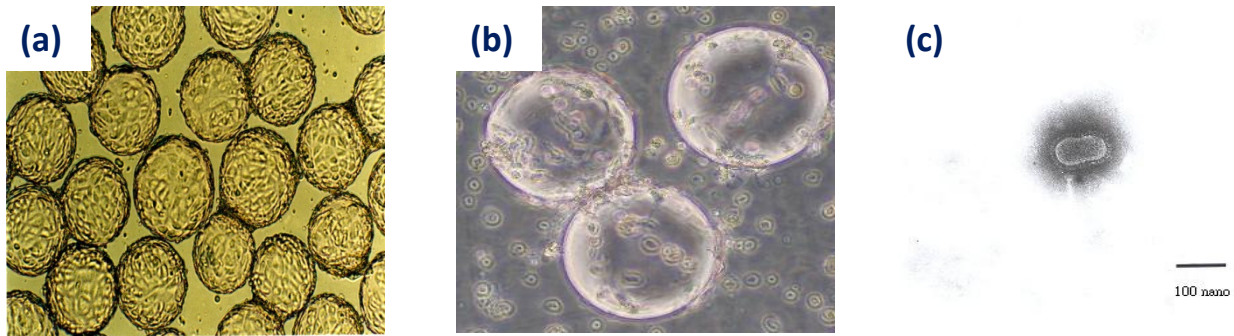
Roller-bottle process flow



Process Development in VRDC



Various scale of microcarrier cell culture bioreactors in Vaccine center.



(a) Confluent microcarriers (b) Cytopathic effect (c) Purified Virus particle .

Single-use bioreactors



Wave bioreactor



Cesco 200L
TideCell



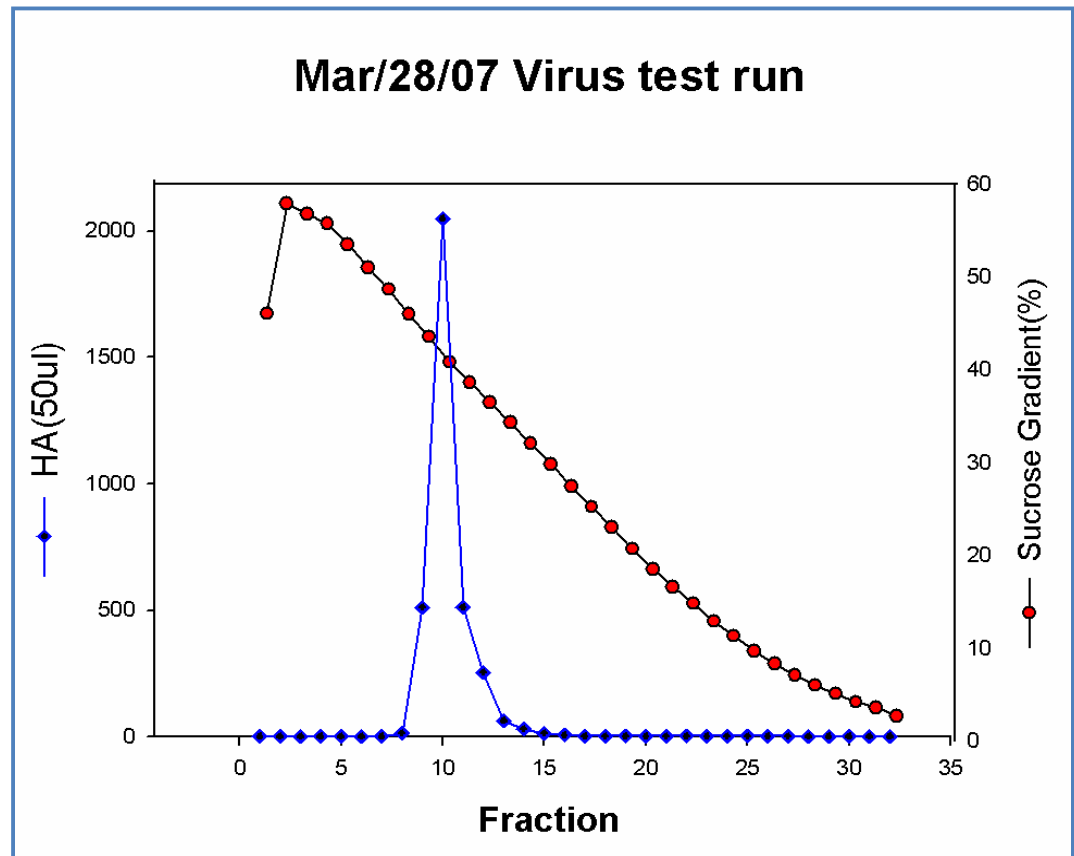
Hyclone 50L/200L SUB



Sartorius 70L Cultibag STR

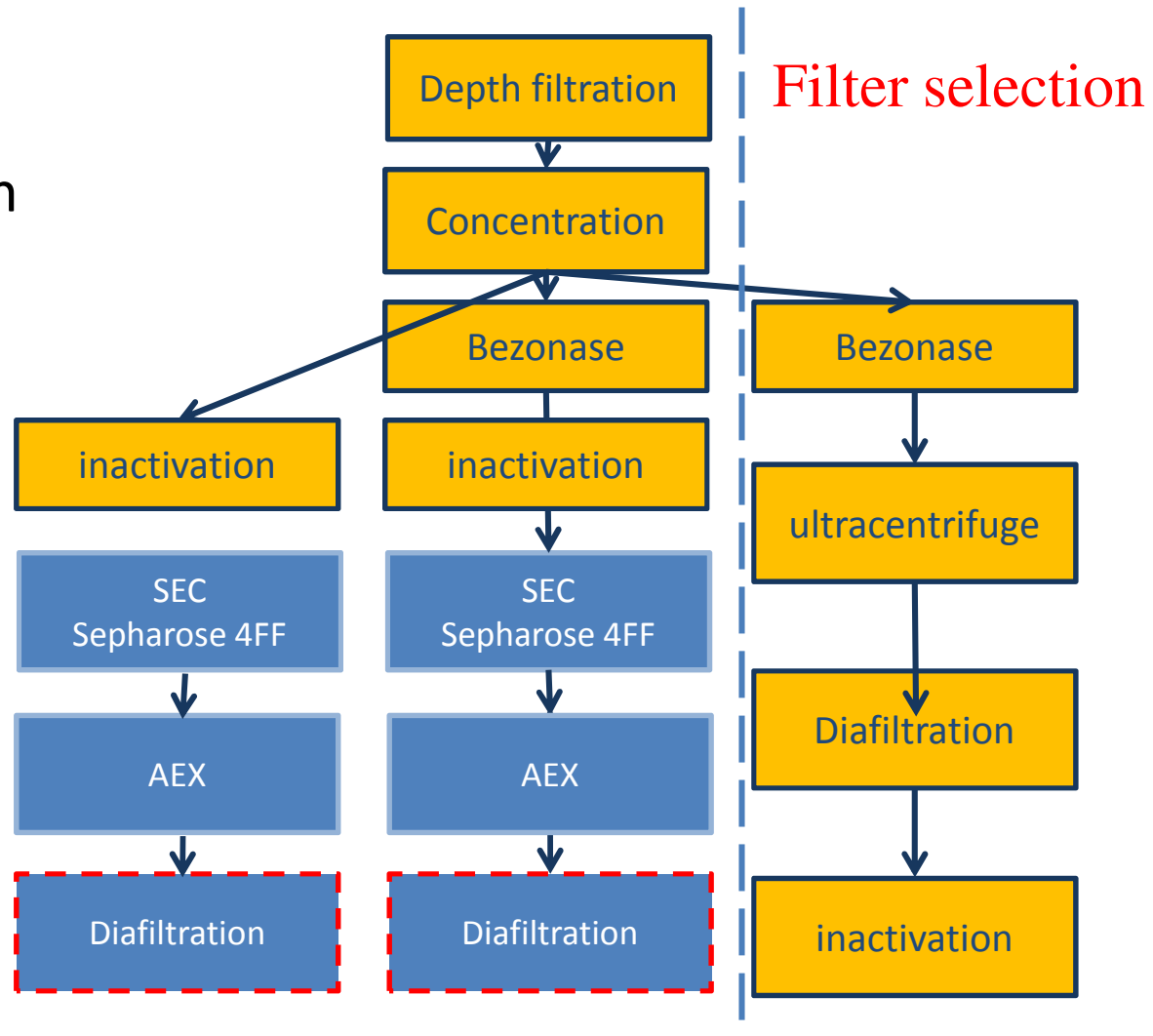


Downstream Purification using Continuous ultracentrifuge

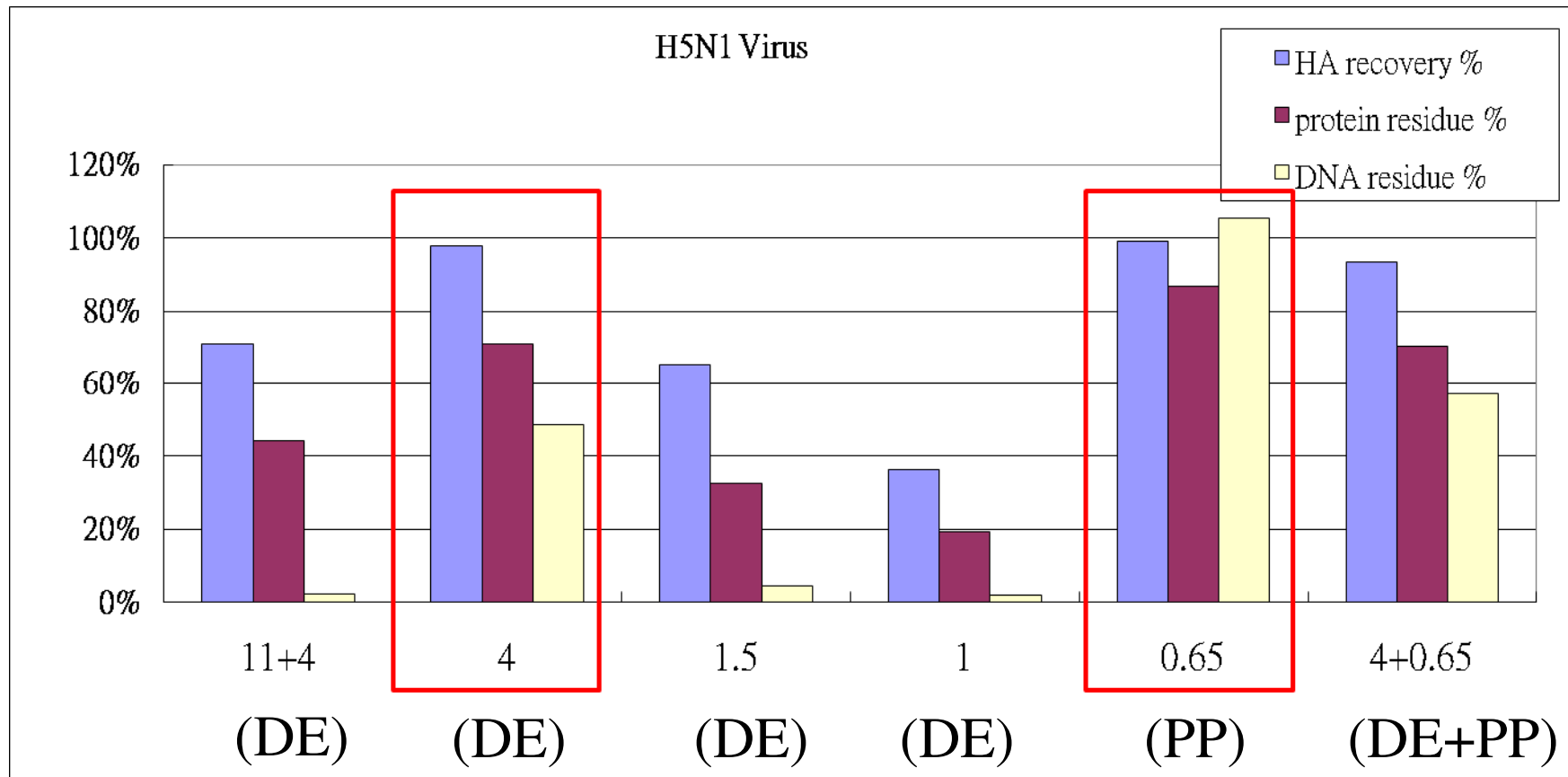


Flowchart of downstream purification scheme

HA yield
residual Protein
residual DNA

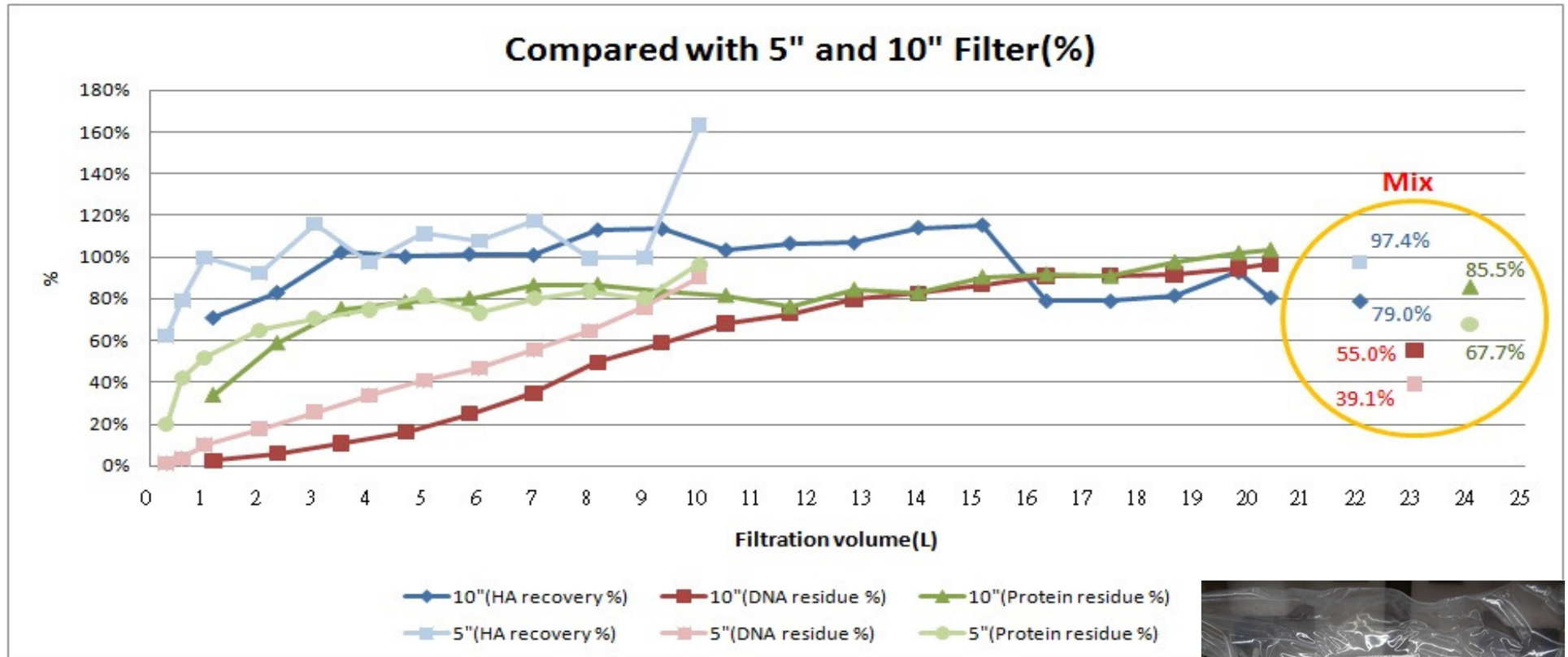


Depth filter selection



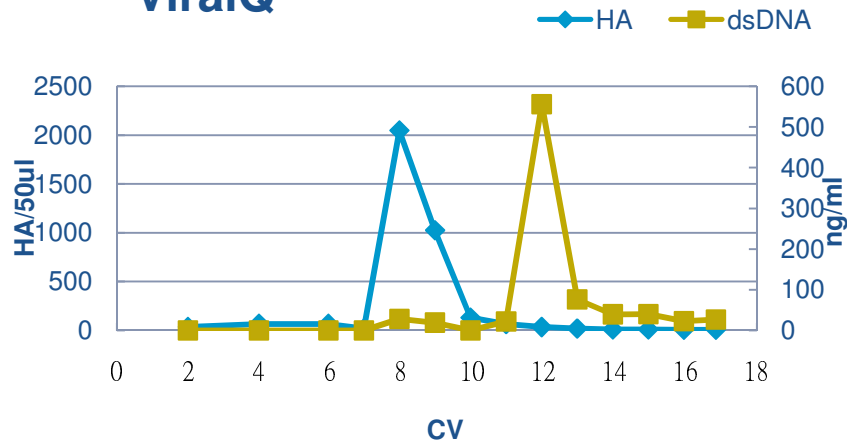
The use of DE: diatomaceous earth (矽藻土) had significant effect on DNA and protein removal

Depth filter test on large scale

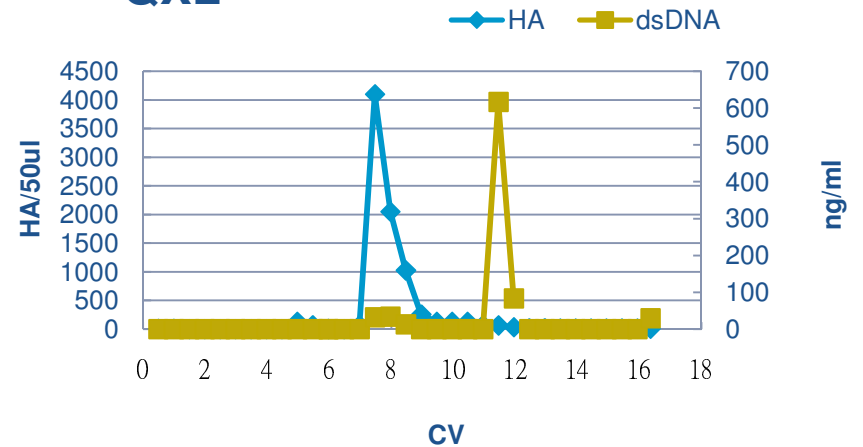


DNA removal by different anion resins

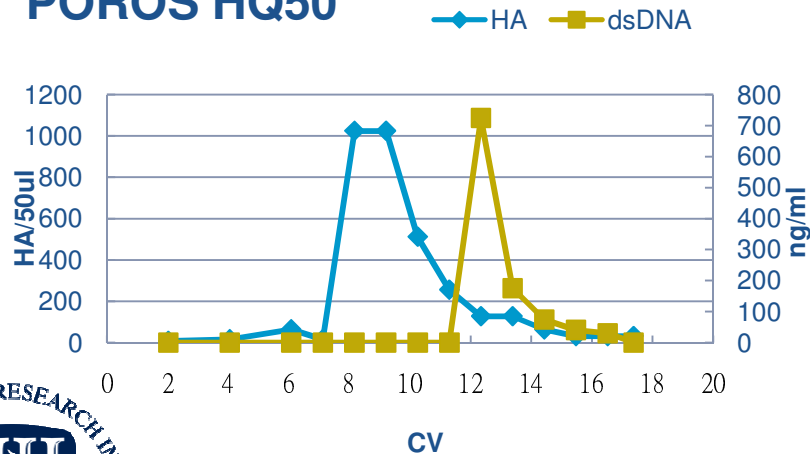
ViralQ



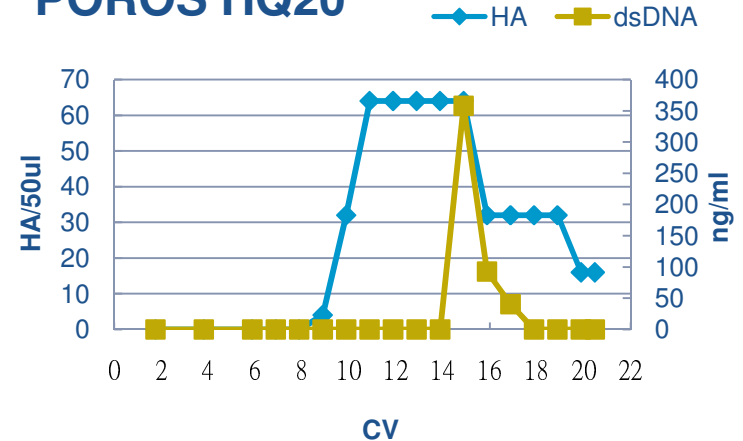
QXL



POROS HQ50

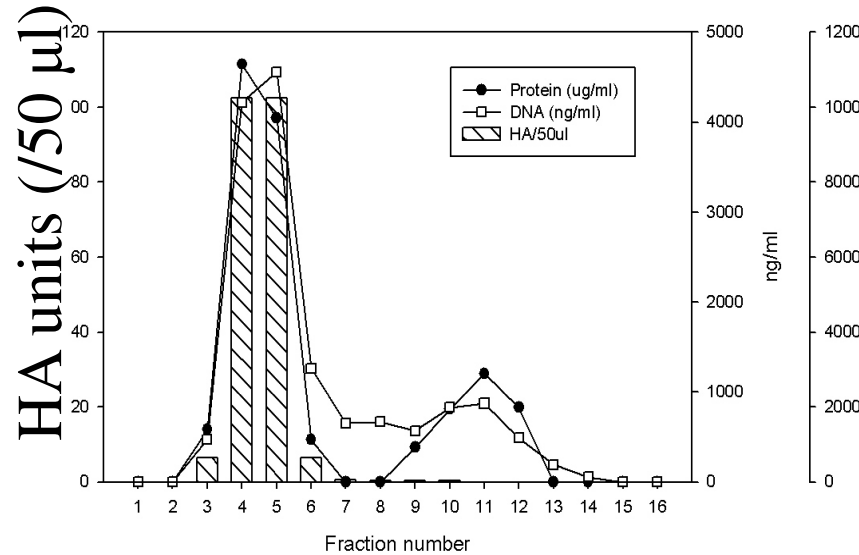


POROS HQ20

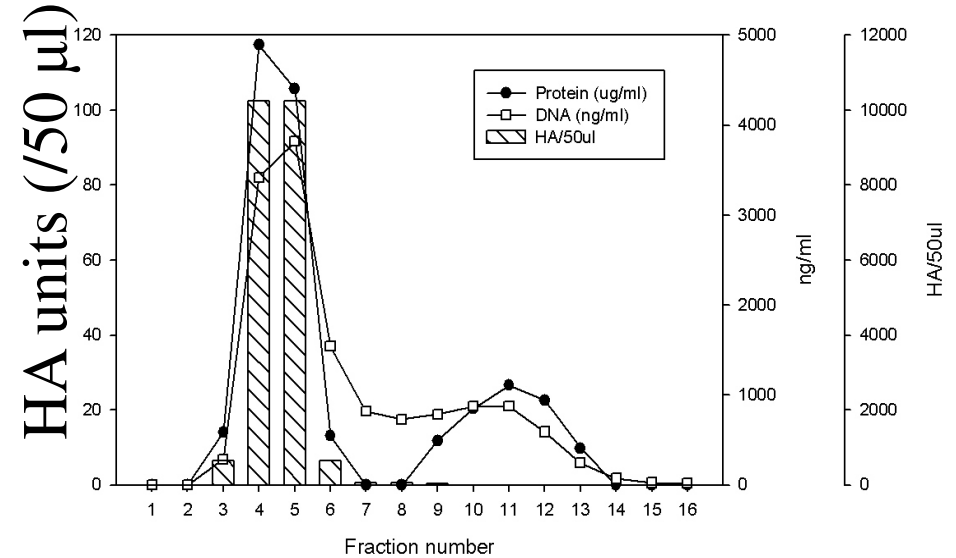


Optimization of size exclusion Liquid chromatography

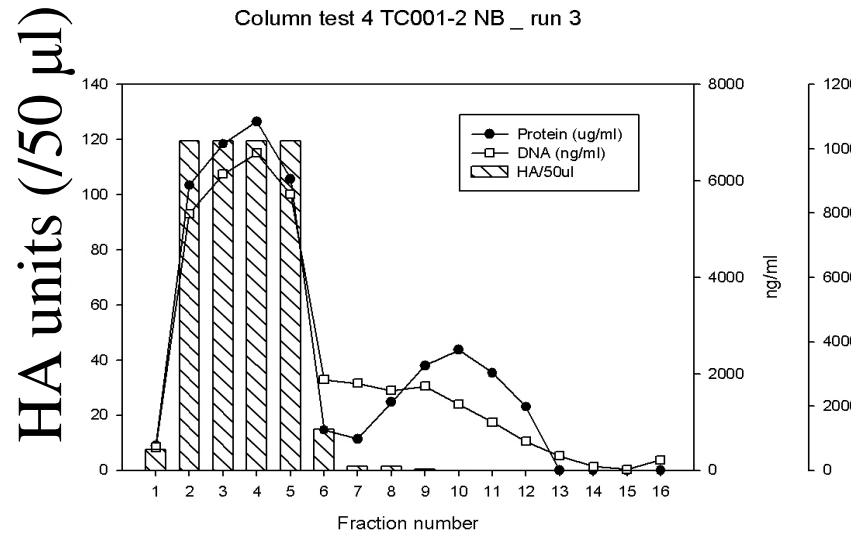
Column test 4 TC001-2 NB_Run 1



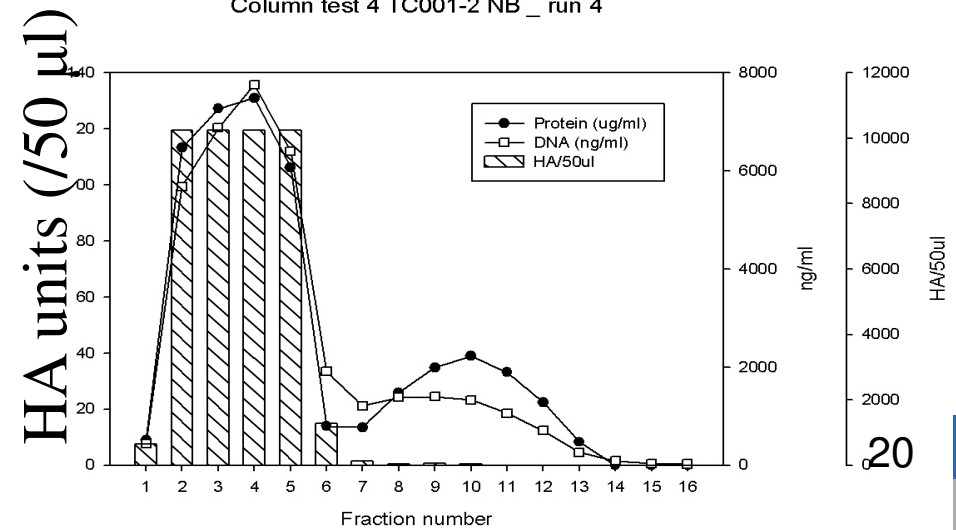
Column test 4 TC001-2 NB_run 2

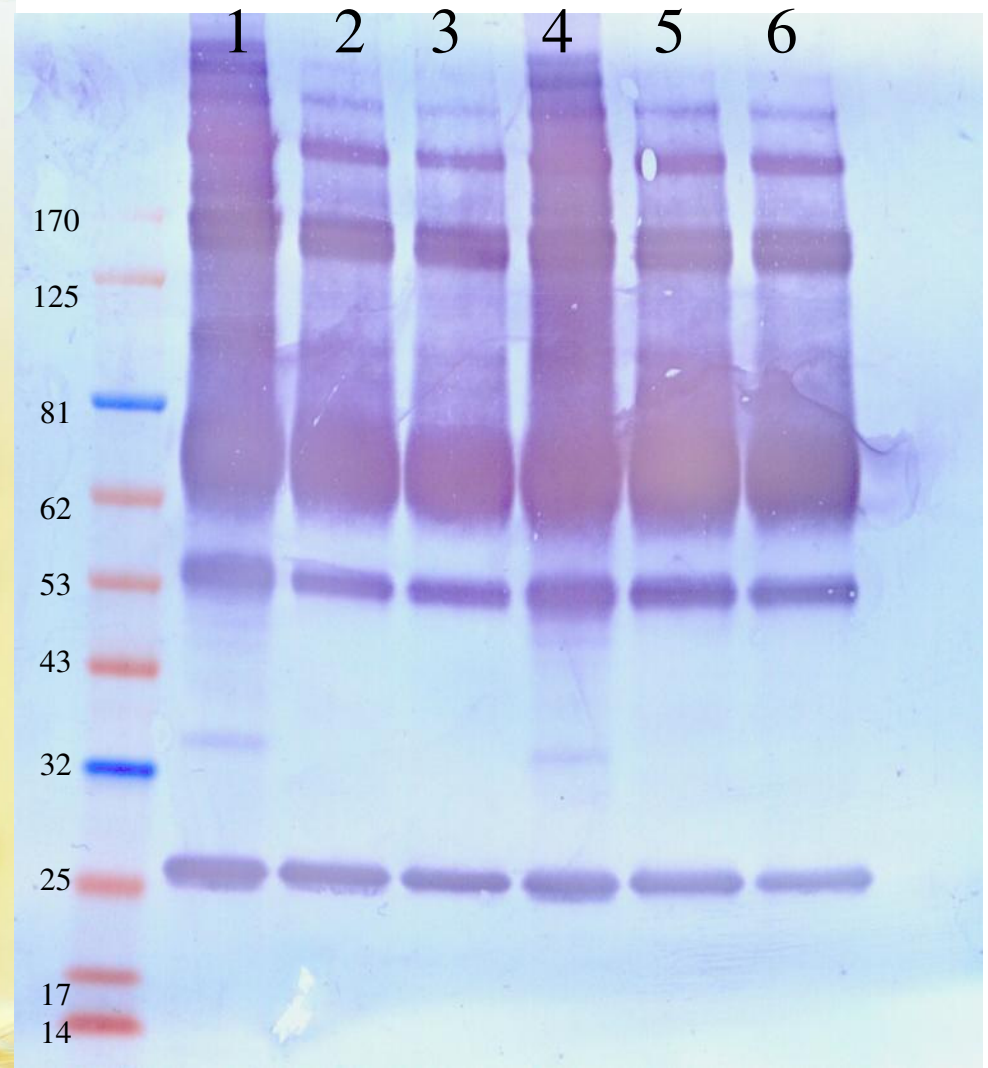
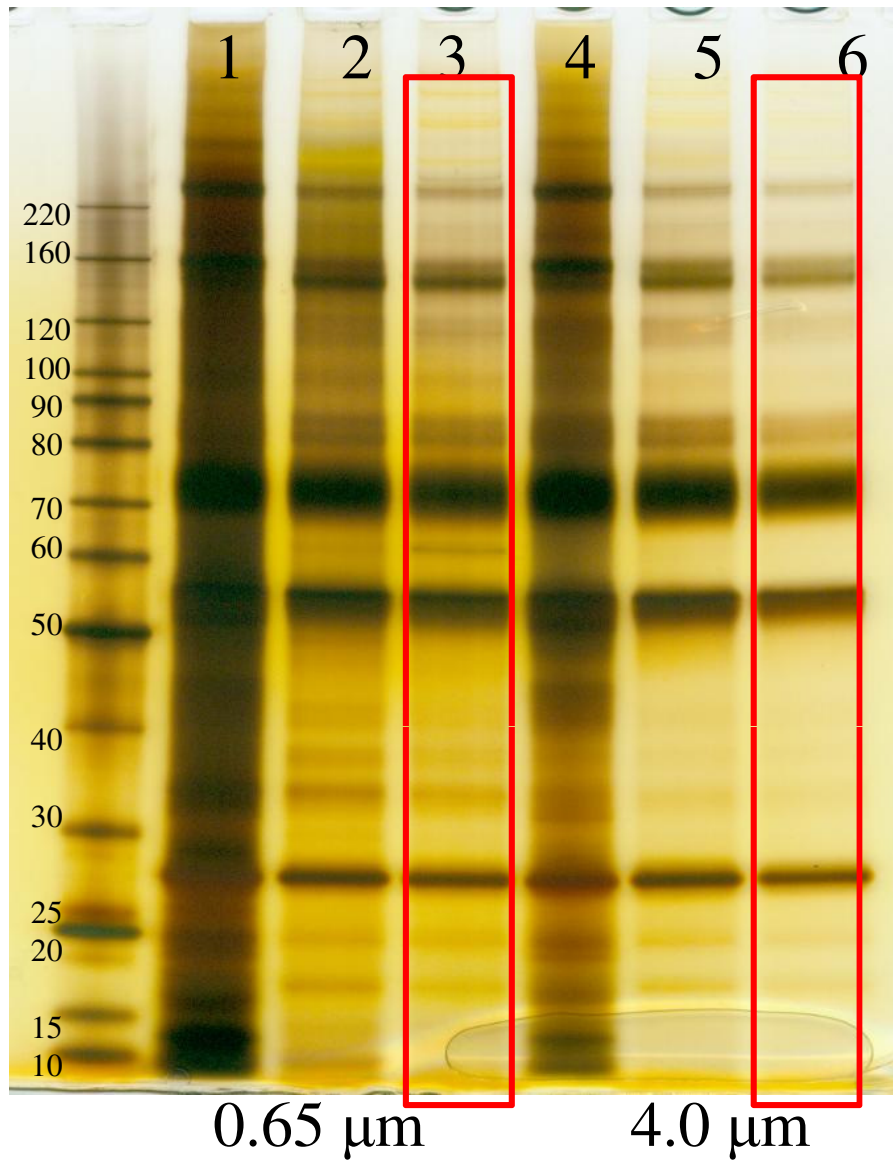


Column test 4 TC001-2 NB_run 3



Column test 4 TC001-2 NB_run 4





Loading: 8 μl /well

1: After Concentration
2: After Gel filtration
3: After Anion-exchange

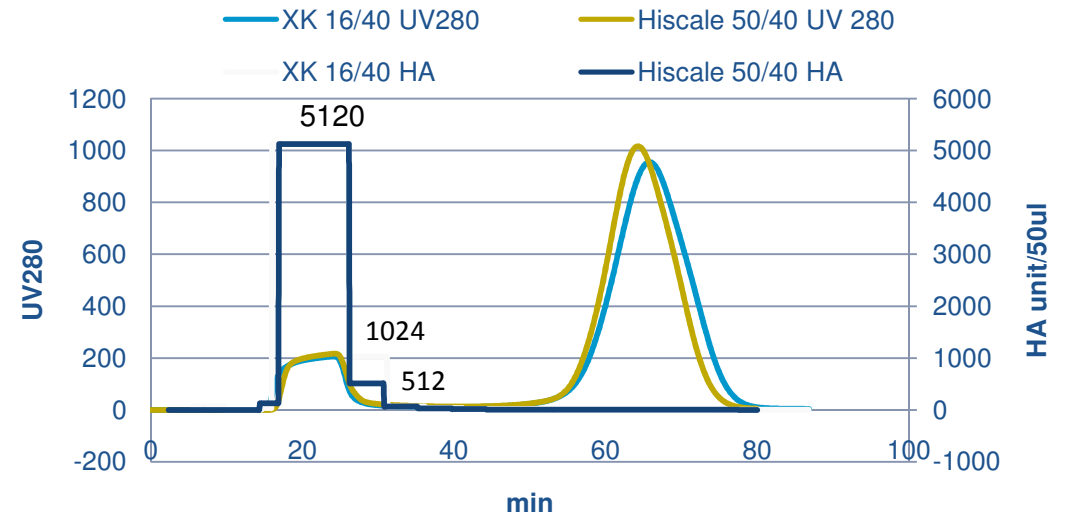
4: After Concentration
5: After Gel filtration
6: After Anion-exchange



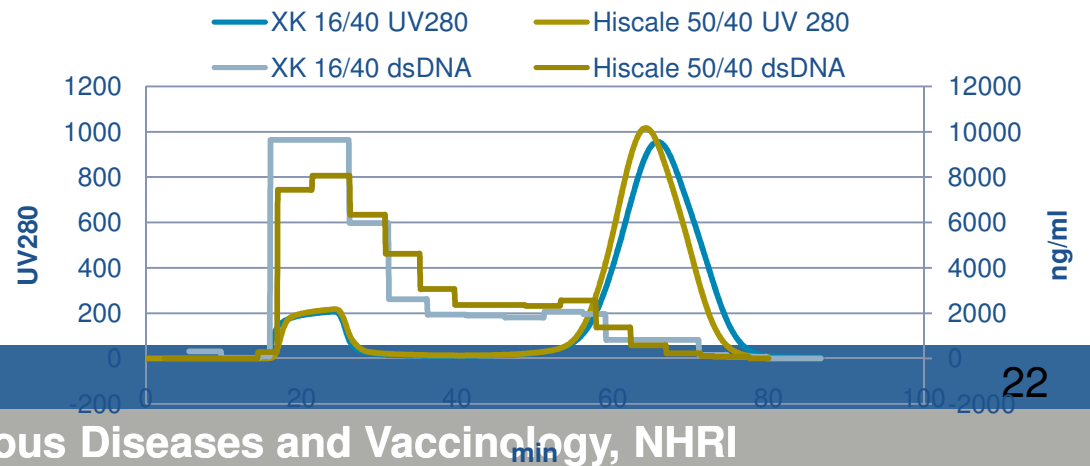
Process scale-up in downstream purification



HA unit



ds DNA concentration



10x scale-up

National Institute of Infectious Diseases and Vaccinology, NHRI

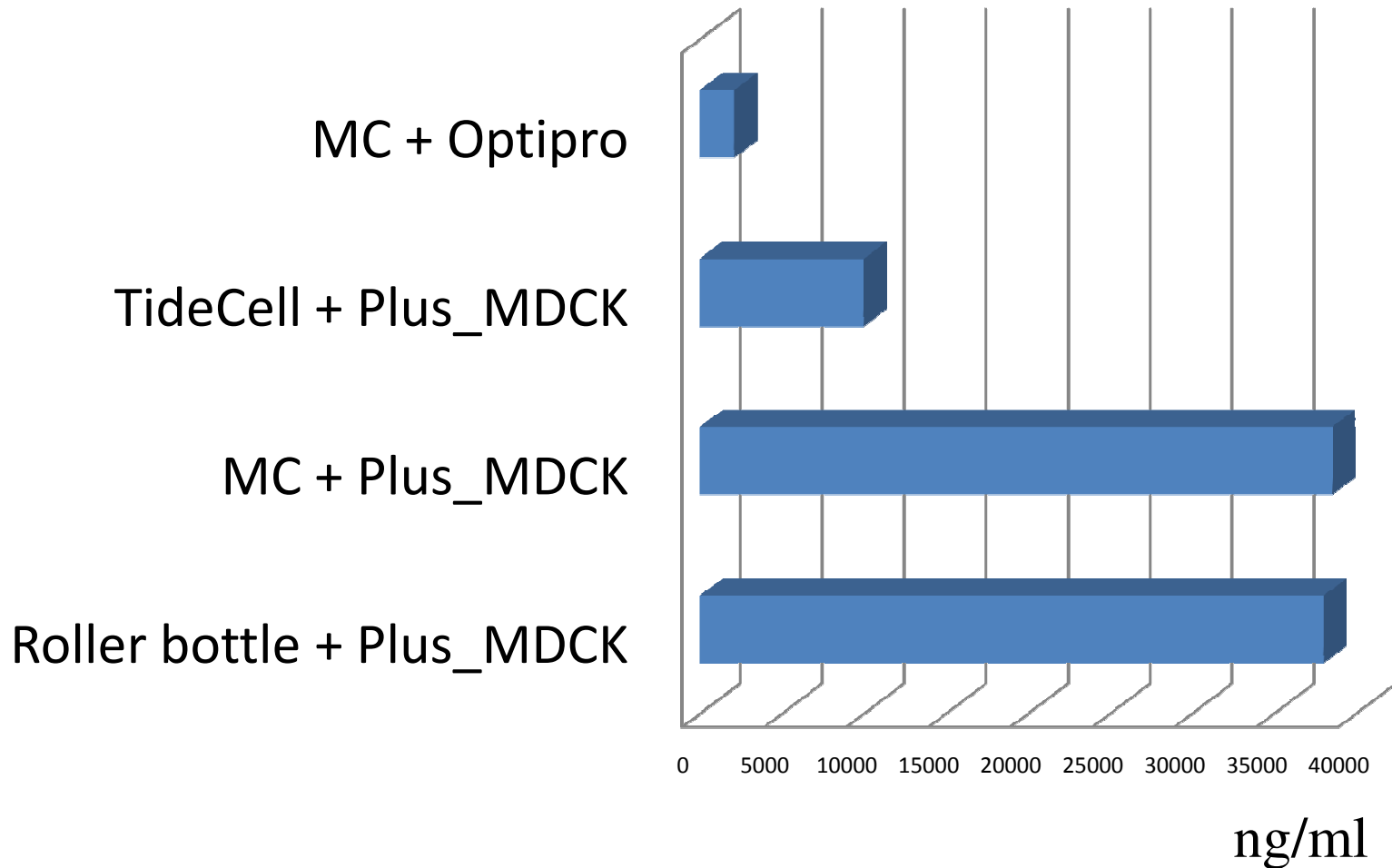
Comparison of purification using different schemes

	\$ 282			\$ 265	
	TC001-1 ultracentrifuge Bulk (0.65)	TC001-1 NB Bulk (0.65)	TC001-1 B Bulk (0.65)	TC001-2 NB bulk (4um)	TC001-2 B bulk (4um)
SRD (ug/ml)	76.1	32.32	33.18	24.67	29.81
BCA (µg/ml)	NA	68.06	69.21	45.56	41.19
µg/dose	NA	30.9	31.5	28.5	20.6
residue DNA (ng/ml)	8.334	10.926	14.006	3.094	2.6
residue DNA (ng/dose)	1.6	5.0	6.4	1.9	1.3
Predicted Doses (based on 4L harvet)	1015	1465	1504	1118	1351

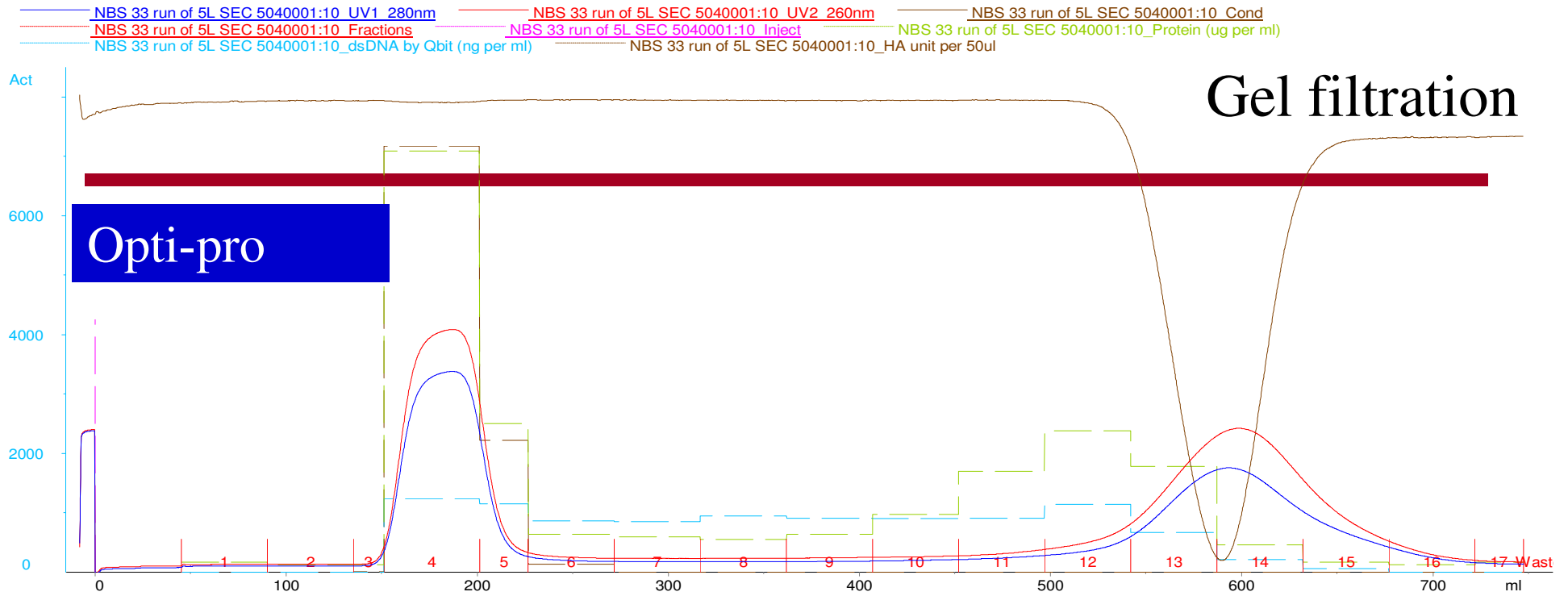
Before 0.2 µm



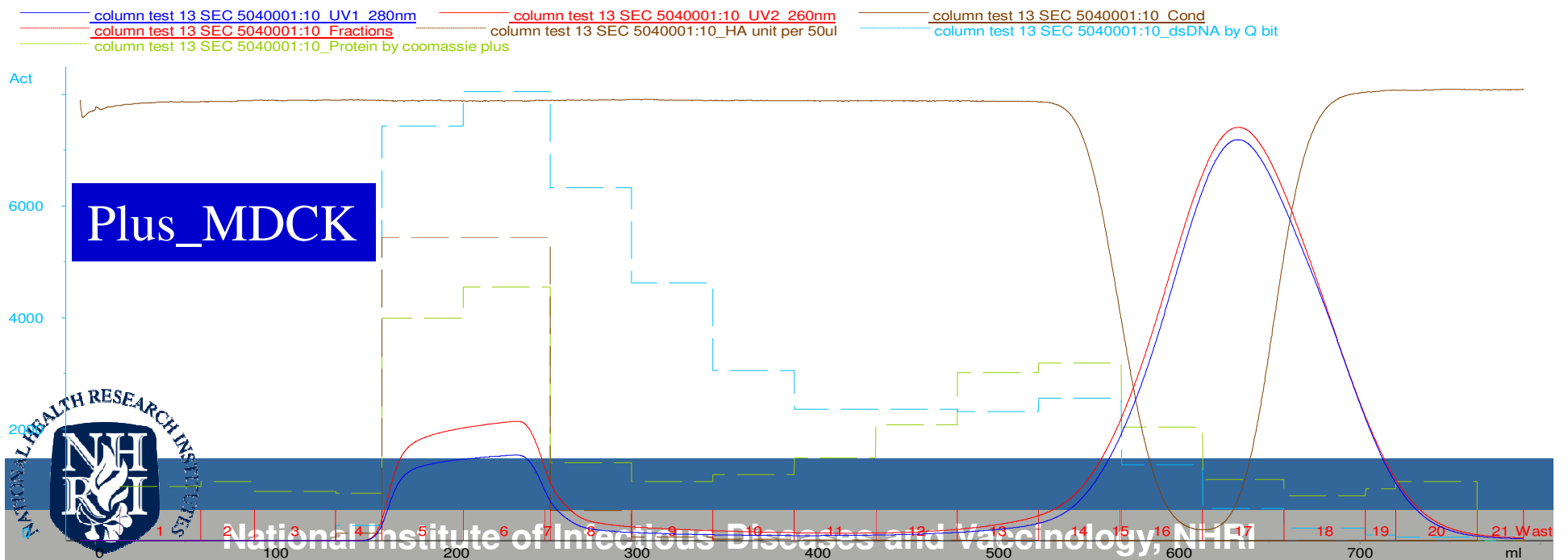
DNA variation from different culturing system



Gel filtration



Opti-pro



Plus_MDCK



Summary

- The use of diatomaceous earth had significant effect on DNA and protein removal, but mechanism is not clear how DE absorbed the impurities
- Low DNA content can be achieved by SEC and AEX columns, thus no addition of DNase is needed
- The use of LC chromatography showed similar results that compared to ultracentrifuge technology
- Variations from Upstream harvest could affect heavily on downstream purification
- New method need to be implemented for improving viral yield



Acknowledgements

- Department of Health
- National Science Council
- Taiwan CDC
- Center for Drug Evaluation
- Science Advisory Board



Bioprocess team

