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Challenges and Solutions for the Next Generation of Vaccines:
Development of Cell Culture-based Live Attenuated Influenza Vaccine

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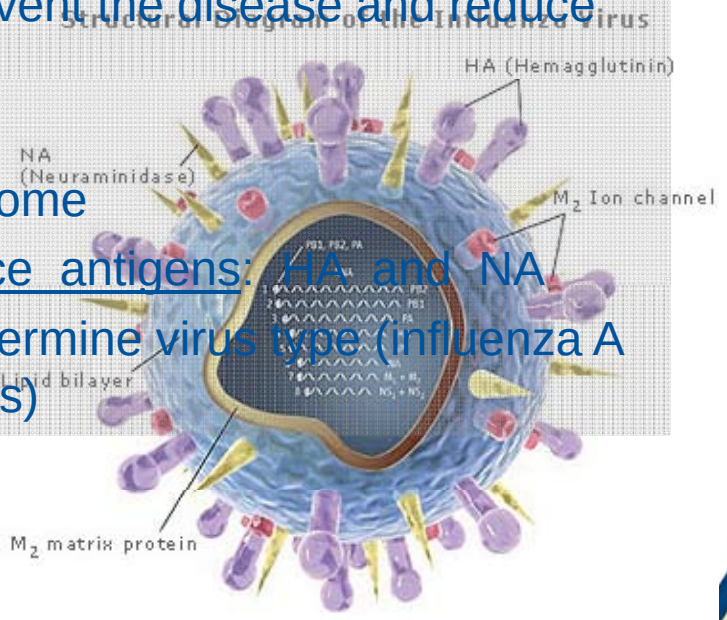
Influenza

- **A Major health problem worldwide**, each year
  - 5-15% of the population are affected with upper respiratory tract infections
  - 3-5 million cases of severe illness
  - 250,000 – 500,000 deaths
- **Pandemic and seasonal flu**
- **Vaccination** - the principal measure to prevent the disease and reduce the impact
- Caused by **influenza virus**
  - 8 segmented negative sense RNA genome
  - encode 11 proteins including 2 surface antigens: HA and NA
  - Antigenic differences in HA and NA determine virus type (influenza A viruses) and lineage (influenza B viruses)



http://www.who.int/mediacentre/factsheets/fs211/en/index.html

Kaiser *et al.*, Science, Vol 312, p. 380

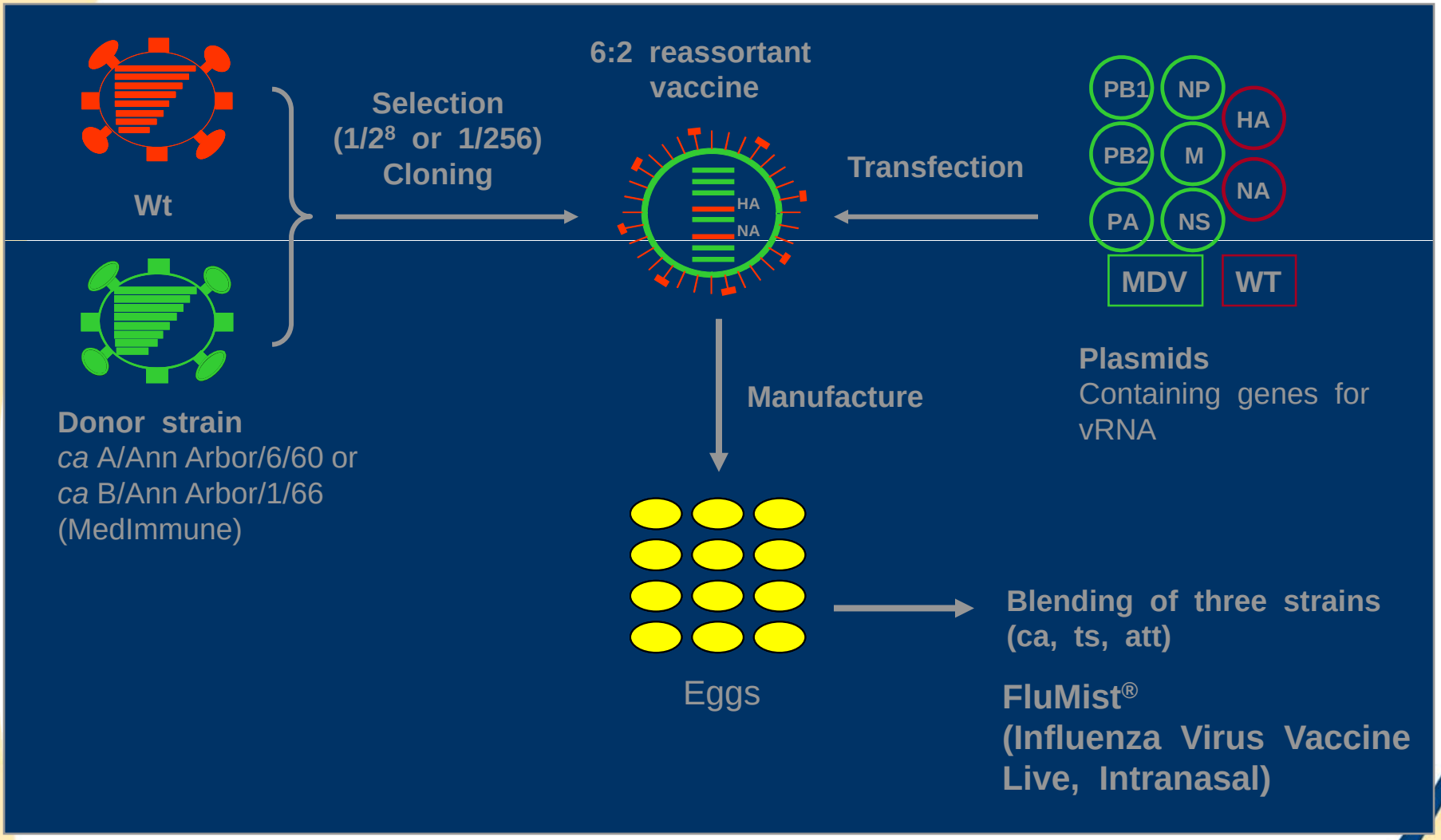
FluMist®

FluMist® (Influenza Virus Vaccine Live, Intranasal)

- Cold adapted, live attenuated vaccine
- Innovative technology (nasal administration)
- Antigen sparing (high yield)
- Durable mucosal and systemic immunity
- High efficacy*

*Belches *et al*., NEJM, 356 (7), p. 685-696

Manufacture of FluMist® Influenza Vaccines



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Old and New Influenza Vaccine Production Technologies

- Challenges with egg production platform
  - Egg stock vulnerability
  - Production capacity limited
  - Less defined biological starting material and significant operator intervention
  - Egg allergies
- Advantages of cell culture-based production platform
  - Susceptibility to a broad spectrum of influenza virus strains
  - Better defined production substrate
  - More controlled manufacturing process sequestered from the environment
  - Surge capacity flexibility
  - Rapid scale-up

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Production Cell:

Madin-Darby Canine Kidney (MDCK) cells

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Selection of Host Cell for Cell Culture Flu Vaccine Production

- Thirteen cell lines (9 mammalian and 4 avian host cells) were tested
  - MRC-5, WI-38, VERO, FRhL-2, 293, NIH 3T3, CHO, MDCK and other human cell lines
  - CEF, CEK, DF-1 and avian embryonic stem cell line
- Only Vero and MDCK cells produced viruses >6.0 $\log_{10}$ $TCID_{50}$/ml for FluMist strains
- Only MDCK cells produced viruses with titer >7.0 $\log_{10}$ $TCID_{50}$/ml for all types and families of seasonal strains tested

Challenges Related with Use of MDCK cells for Vaccine Production

- Original line of MDCK cells was non tumorigenic
- Some MDCK derivatives have been found to be highly tumorigenic
- Highly tumorigenic cell substrates have never been used to manufacture viral vaccines
- Highly tumorigenic cell substrates pose significant regulatory challenges

Krause, VRBPAC 2005

Product Safety and Regulatory Concerns !

MedImmune's Approach to Minimize Risk of MDCK Cells

- **Produced cell bank with low tumorigenic potential from biologically cloned cells**
- Extensive testing strategy developed Process developed to deliver vaccine with the following characteristics
  - Sterile product
  - Acellular
  - Reduction of host cell DNA quantity
  - Reduction of host cell DNA size
  - Minimal exposure to animal derived components (ADCs)
- with guidance from CBER for:
  - Adventitious agents
  - Tumorigenicity of live, intact cells
  - Oncogenicity of host cell DNA and lysate

Produced Low Tumorigenic Cell Bank

- Development of Serum-free MDCK Cells

- Numerous in-house SFM formulations developed
- Cells evaluated for several primary factors
  - Maintenance of cell line growth
  - Potency (influenza virus yield)
  - Karyology
  - Tumorigenicity in athymic nude mouse model

Tumorigenicity of 10^7 Uncloned MDCK Cells in Adult Nude Mice

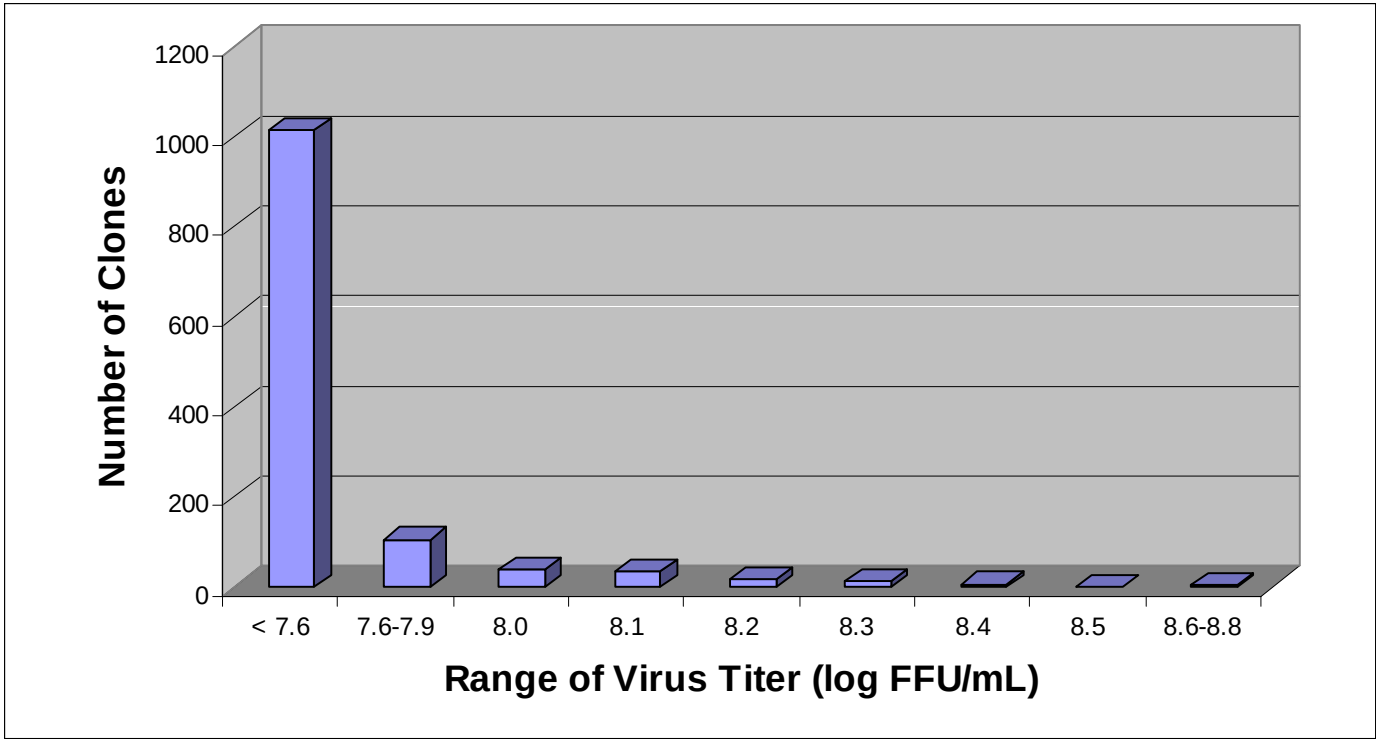
Treatment Group (n=10)	Animals with Tumors	Tumor Formation Rate	Note
MDCK Cells in Serum medium	0	0%	
MDCK Cells in SFM A	6	60%	tumors at injection site
MDCK Cells in SFM B	0	0%	
Negative Cell Control	0	0%	
Positive Cell Control	10	100%	tumors at injection site

Produced Low Tumorigenic Cell Bank

- MDCK Cells Cloning

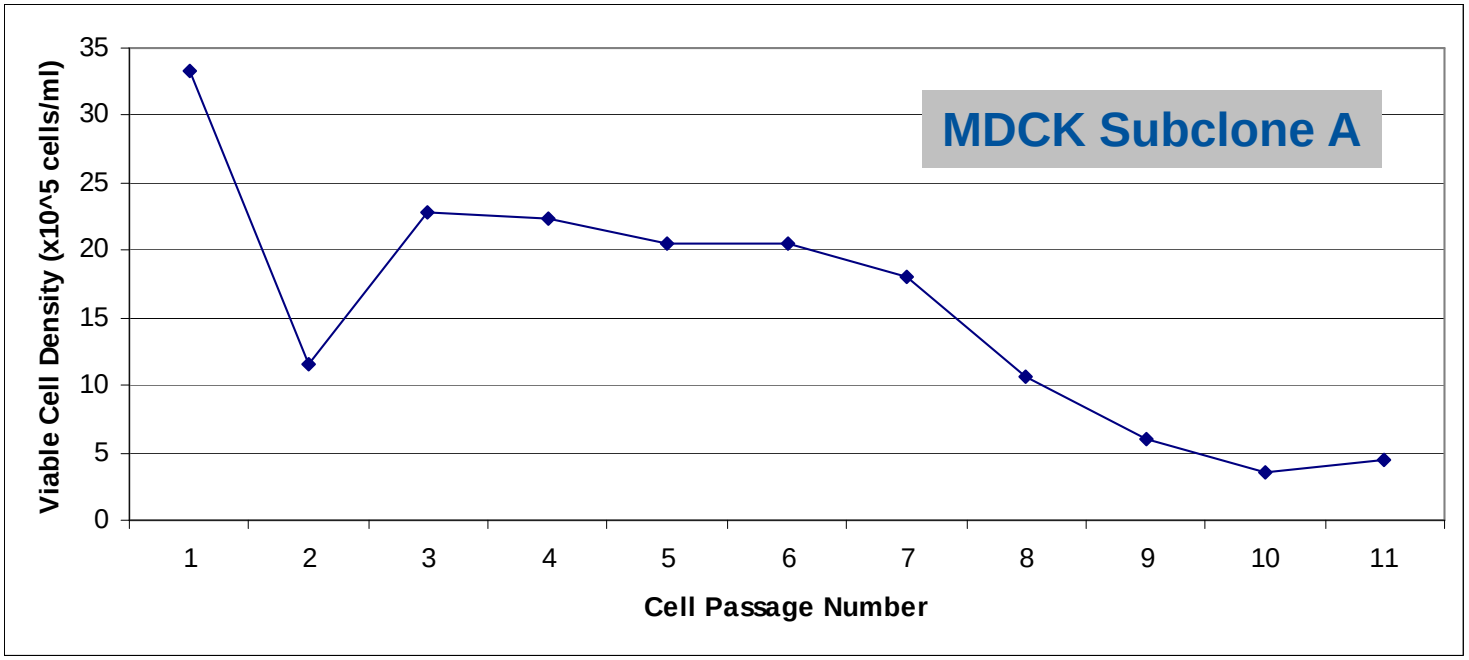
- Began after development of SFM that maintained cell karyology and low potential tumorigenic nature of the ATCC MDCK cells
- Initiated from a new vial of ATCC MDCK cells
- Performed in serum-containing media
- Two rounds of limiting dilution cloning completed
- Clones initially selected based on productivity

Initial Cloning of MDCK Cells



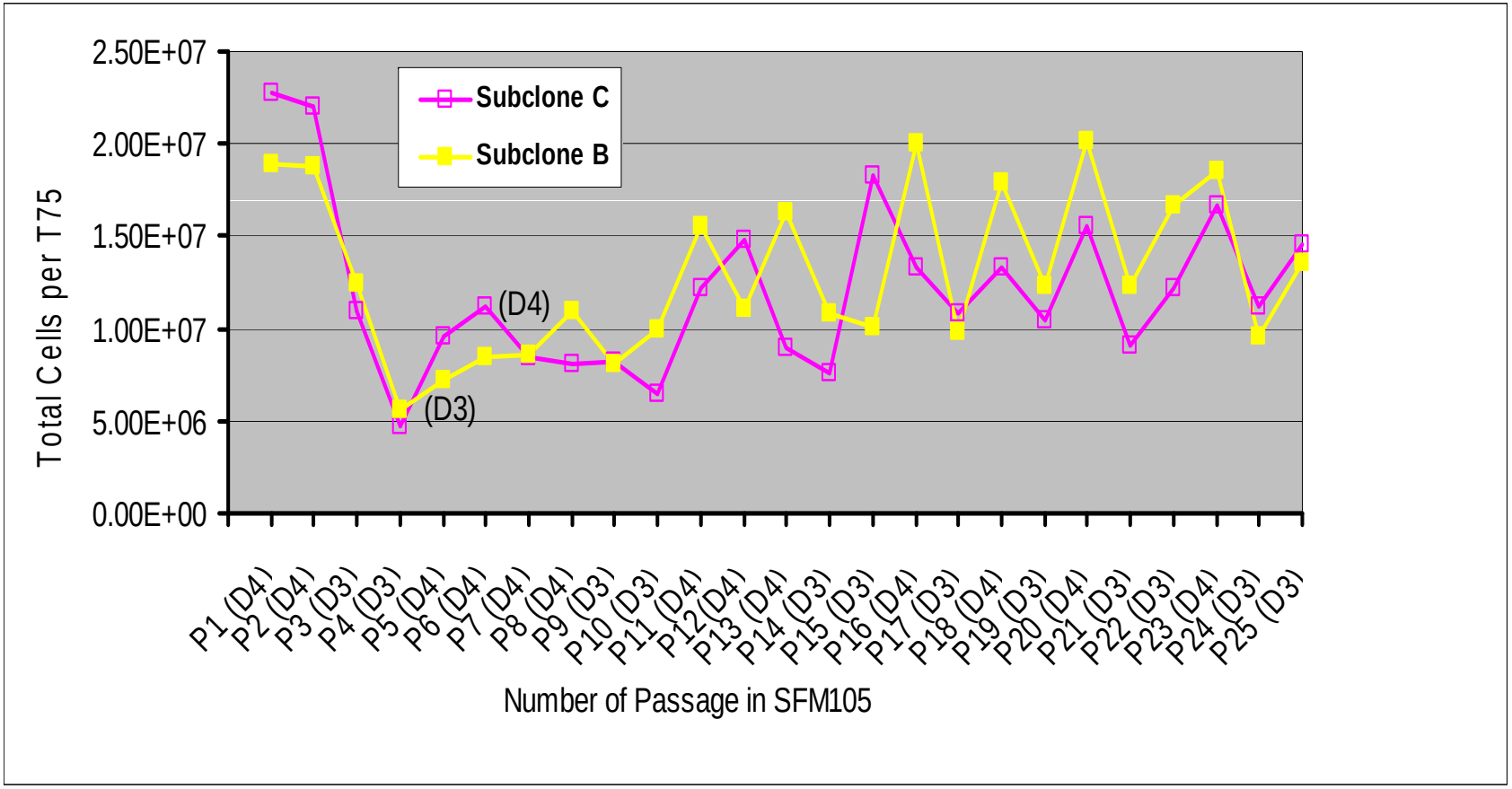
Range	< 7.6	7.6-7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6-8.8	< 7.6 to 8.8
Clone No.	1014	105	38	35	16	11	6	1	2	1228
Percentage	82.6%	8.6%	3.1%	2.9%	1.3%	0.9%	0.5%	0.1%	0.2%	100%

Not All High-Producing Clones in SFM Maintained Cell Growth



- Gradually reduced growth rate in SFM
- No longer growing after 11 passages
- Eliminated from clone selection

Extended MDCK Cell Growth in SFM



Tumorigenicity and Oncogenicity of Cloned MDCK Cells

Study	Test Sample	Animal with Tumors/ Total Animals	Location of Tumors
Tumorigenicity adult nude mice	DPBS	2/33	lymph node, spleen, liver
	Hela cells	38/41	site of injection
	10^5 MDCK cells	1/44*	spleen, liver, lung
	10^1, 10^3,10^7 MDCK cells	0/132	
Tumorigenicity NB nude mice	Hela cells	44/44	site of injection
	10^1, 10^3, 10^5, 10^7 MDCK cells	0/176	

* tumors confirmed by histology examination, not sharing MDCK cell morphology, not located at SOI and not related with MDCK cells as verified by immunohistochemistry analysis

Test Samples	Newborn Animals	non-injected	Saline	MDCK cell lysate	MDCK cell DNA
MDCK Cell Lysate Oncogenicity	**mice**	1/25	0/45	0/45	n.a.
	hamsters	0/25	0/45	0/45	n.a.
	rats	0/25	0/45	1/45*	n.a.
MDCK Cell DNA Oncogenicity	**mice**	0/25	0/45	n.a.	1/45*
	hamsters	0/25	0/45	n.a.	0/45
	rats	0/25	1/45	n.a.	0/45

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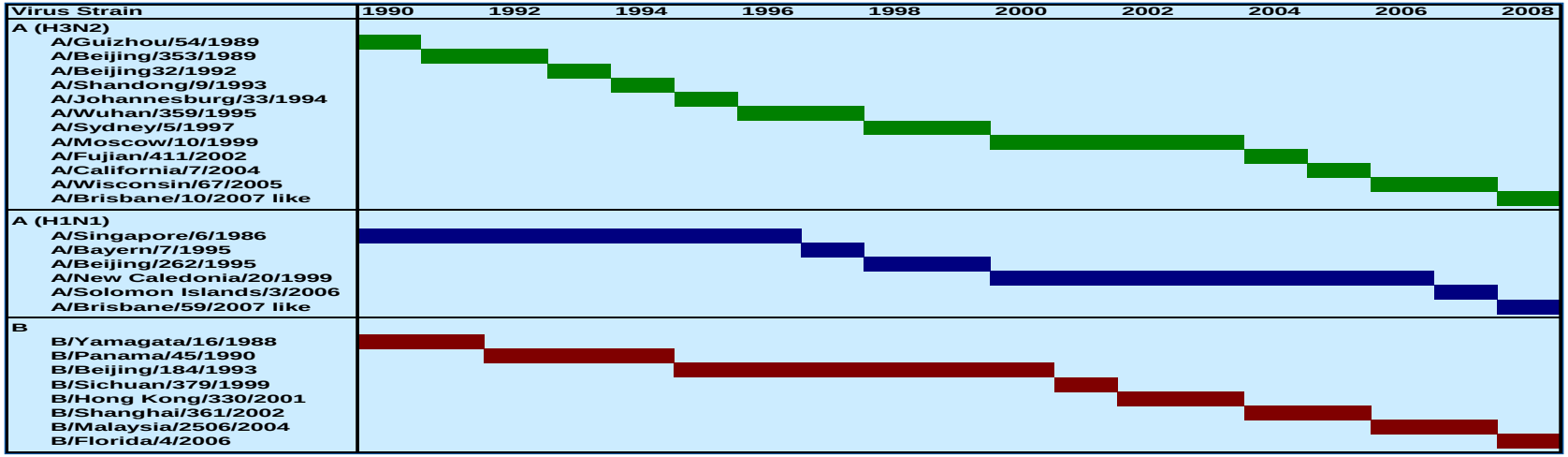
Production Platform:

A Flu Vaccine Manufacture Process without Extensive Process Development

Challenges Related with Annual Flu Vaccine Production

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Surveillance												
Selection												
Reassortment												
Manufacturing												
Distribution												
Immunization												

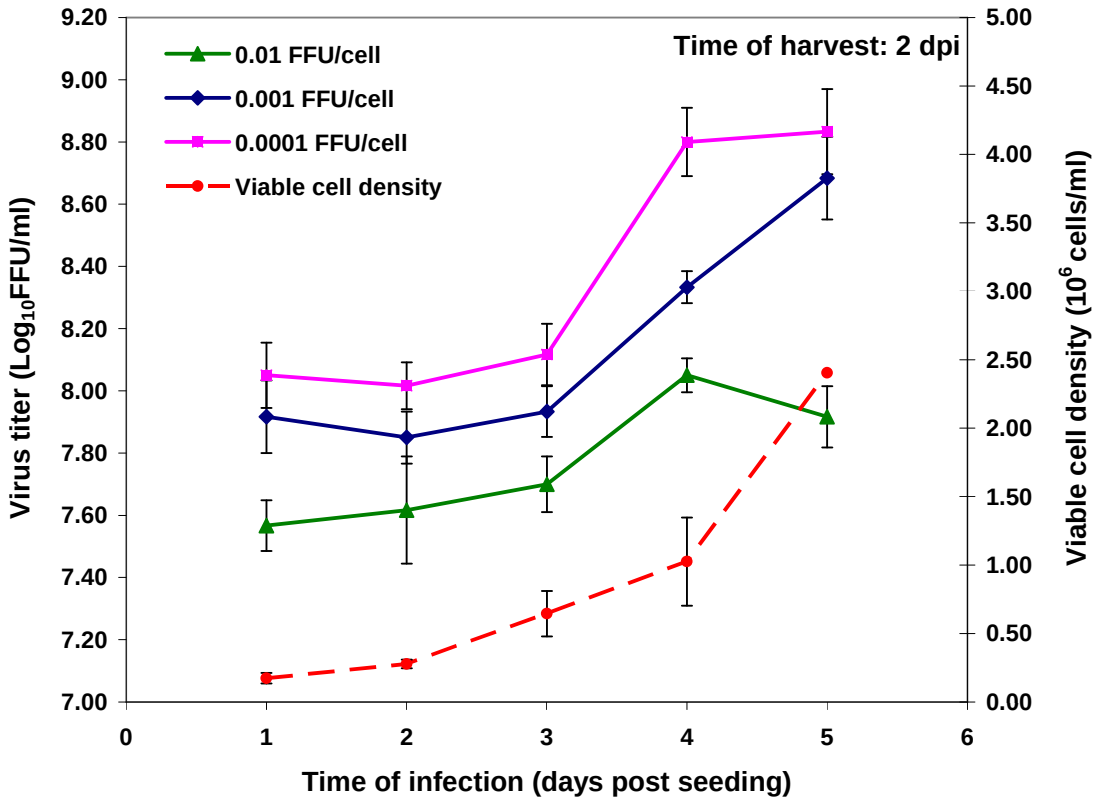


- **Frequent vaccine strains and manufacture process changes**
- **Short (often 1-3 weeks) Process Development Time**

MedImmune's Approach to Address Process Development Challenges

- Understand process parameters to all strains
- Develop a true "platform process" capable of production of all strains
- Conduct DOE and reduce pre-production PD time to approx. 2-4 weeks

Time of Infection and Virus Yield



Virus: A/Wisconsin/67/05

Data collected using shake flasks

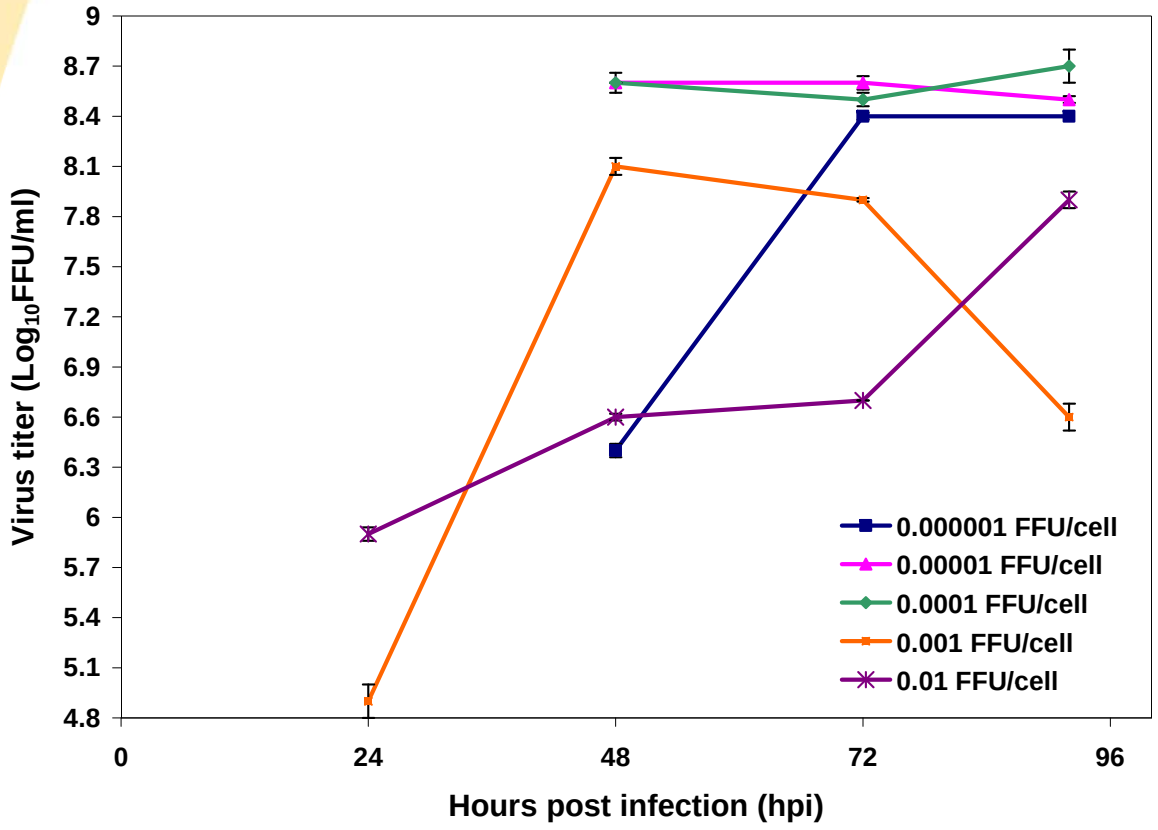
Virus titer estimated using FFA and viable cell density estimated using nuclei counts

Error bars correspond to assay replicates and biological replicates

Peak titer increases with the time of infection (e.g., 4dps vs 3dps) irrespective of the virus input at the time of infection

Trends are similar among different sub-types

Input Virus and Virus Yield



Virus: A/Wyoming/03/03

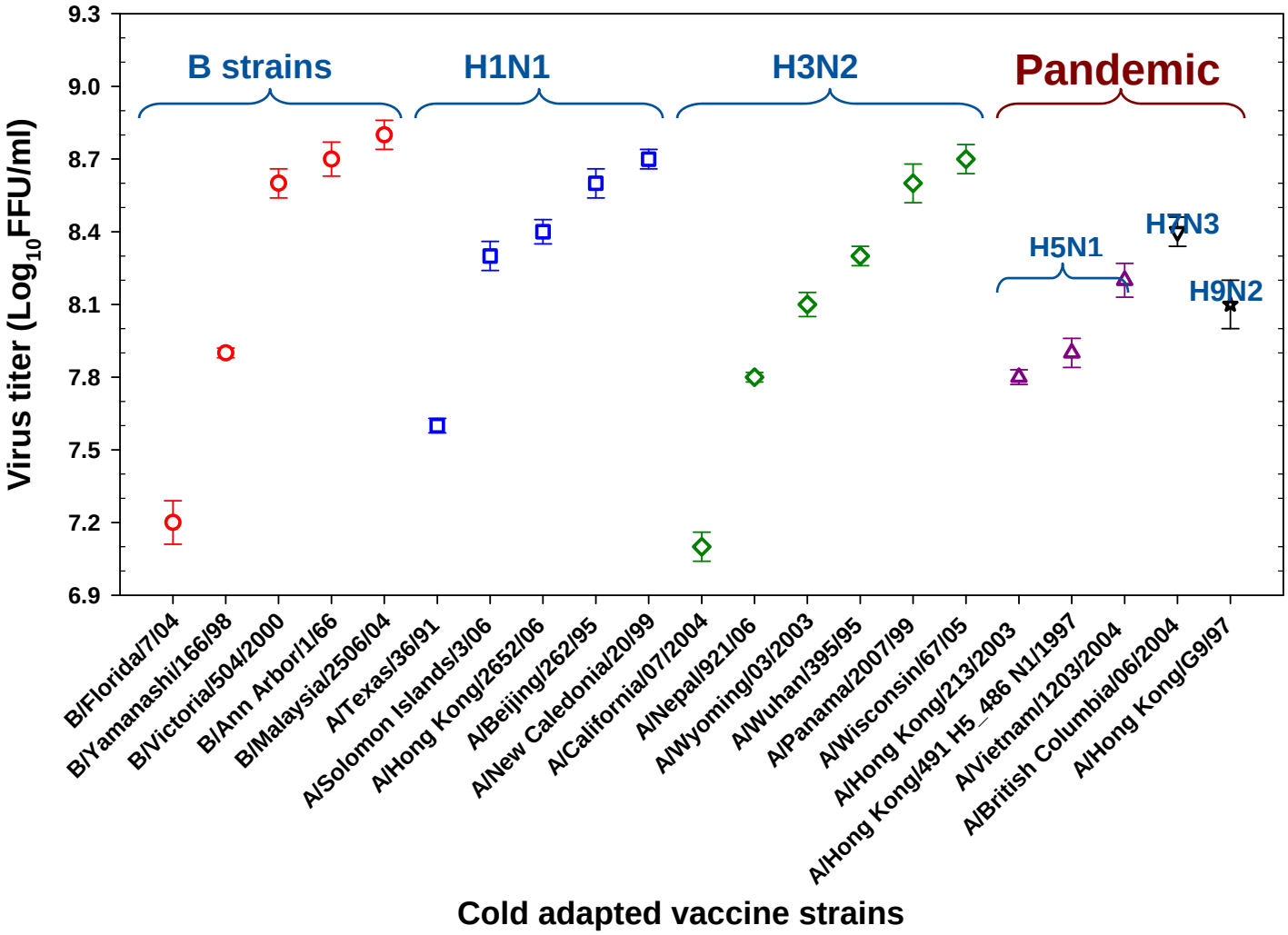
Data collected using shake flasks

Virus titer estimated using FFA and viable cell density estimated using nuclei counts

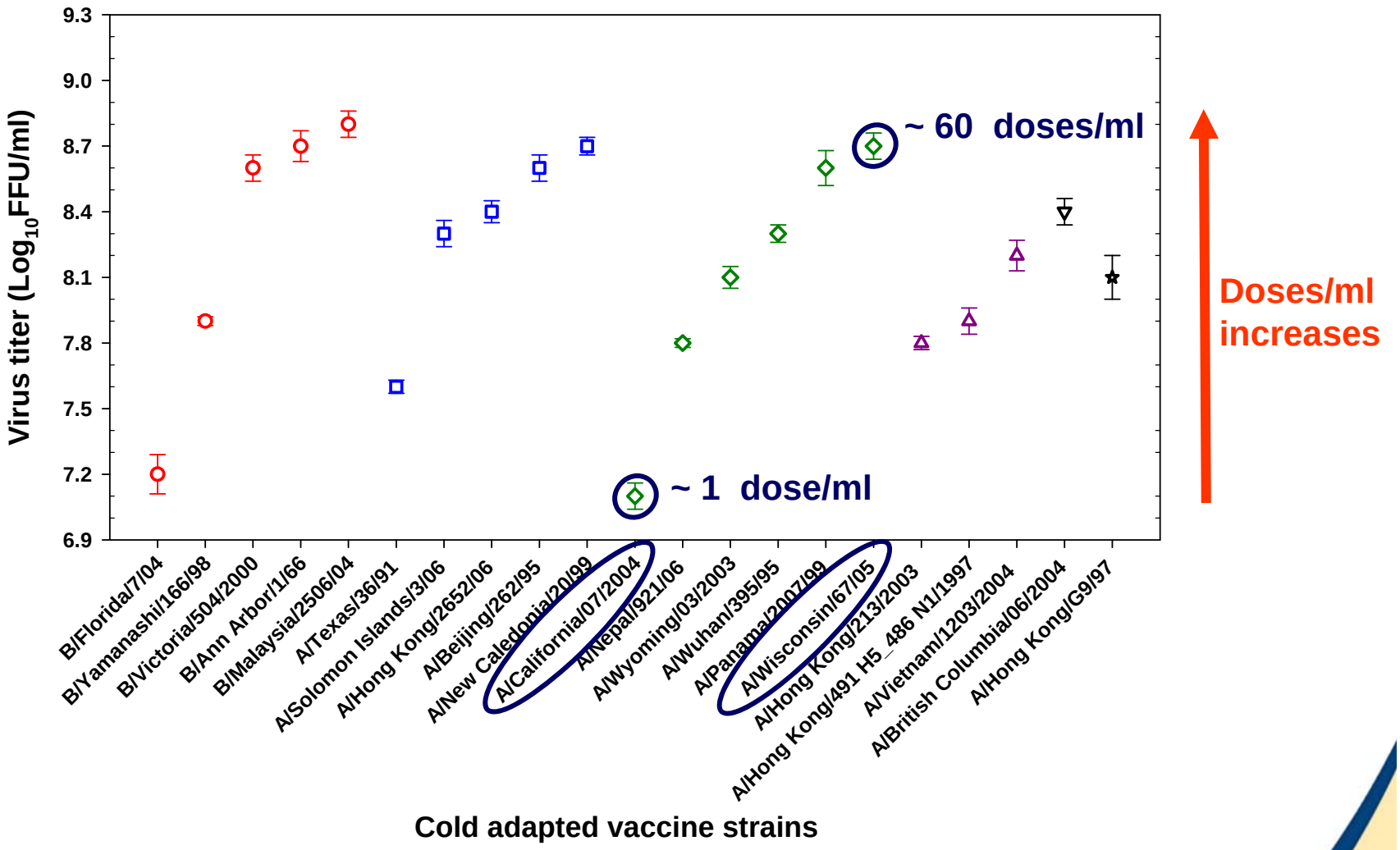
Error bars correspond to assay replicates

Lower virus input improves virus titer

Spread of Virus Productivity Using Phase I Platform Process

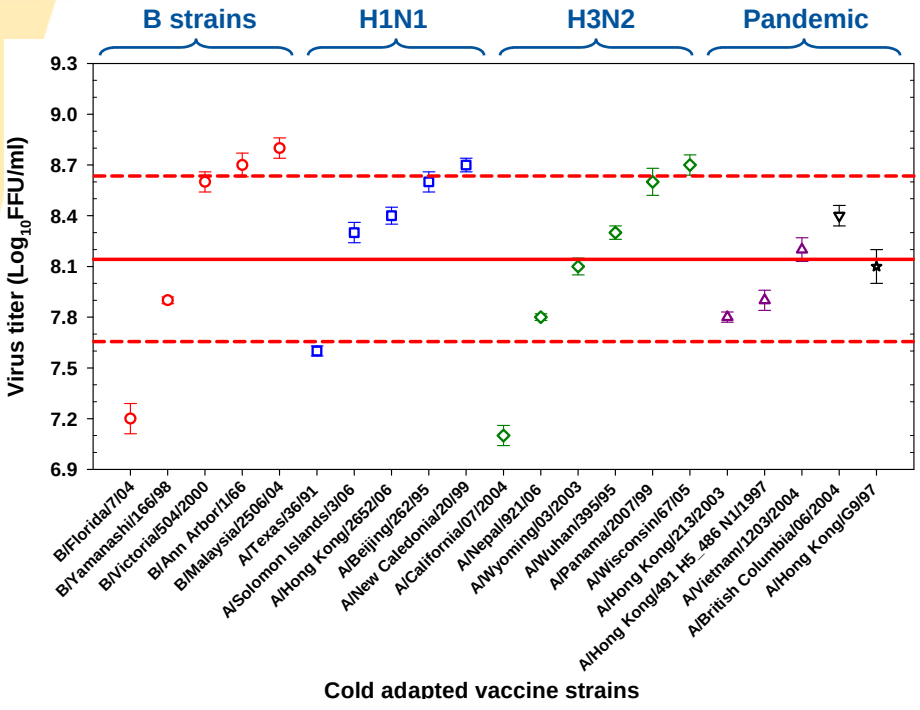


Risks Posed by Virus Productivity Variation

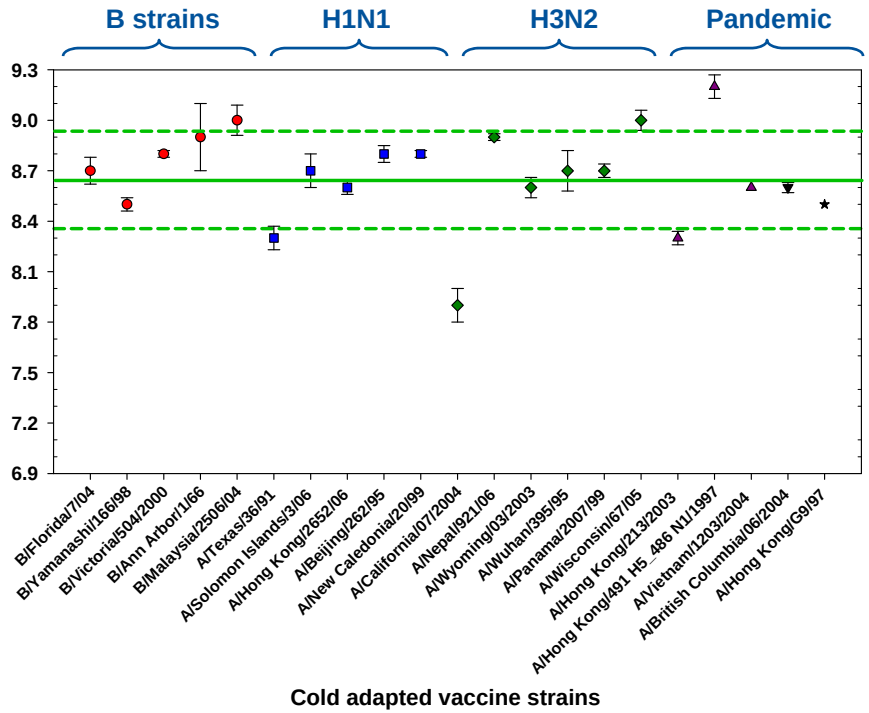


Improvement in Process Performance and Robustness

Pre-optimization

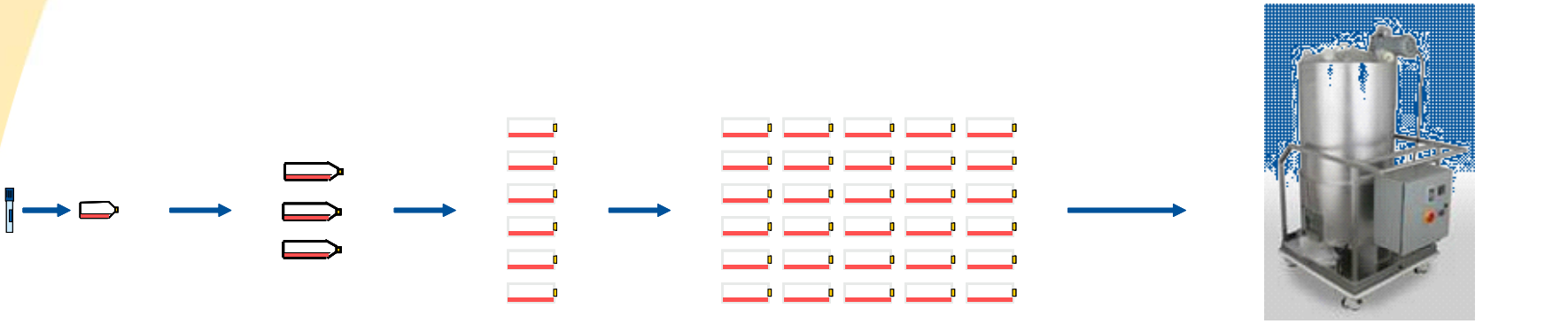


Post-optimization



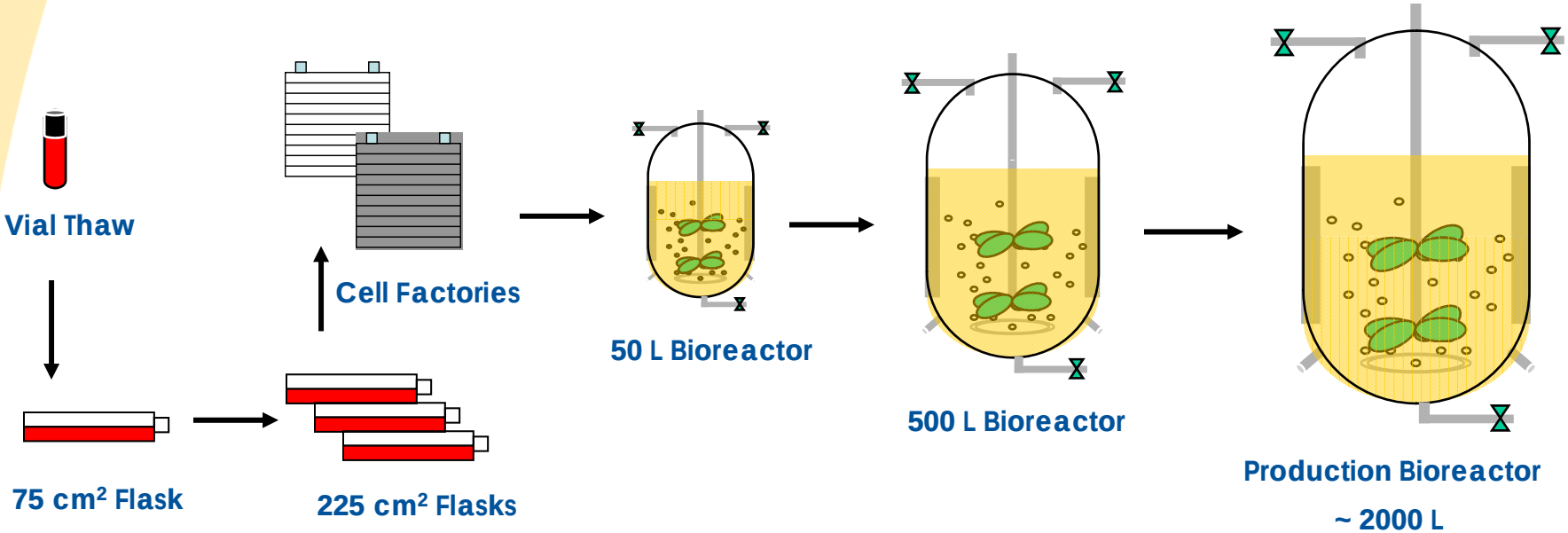
Average productivity improvement = 0.5 log_{10}FFU/ml
Lowest virus titer observed 7.9 log_{10}FFU/ml (up from 7.1 log_{10}FFU/ml)
Reduced variability in yield, increasing process robustness

Fully Disposable Small Scale Platform Process



- **Fully disposable process implemented in GMP Pilot Plant**
  - **No need for cleaning/validation with disposable culture vessels**
  - **Shortened timeline for implementation in clinical production**
- **Quick turned-around between batches (in a few hours), making possible to re-start production very rapidly.**

Projected Large-scale Manufacturing Process



Theoretical Bulk Vaccine Dose Output

Purification Yield (%)	Number of Trivalent Doses (x10E+6)					
	Harvest Titer (FFU/mL)					
	8.0	8.1	8.2	8.3	8.4	8.5
15	58.7	73.9	93	117.1	147.4	185.6
20	78.2	98.5	124	156.1	196.5	247.4
35	97.8	123.1	155	195.1	245.7	309.3

Trivalent doses for 2x2000L bioreactors, 6 month campaign, 7.0 $\log_{10}$ FFU dose

Summary and Outlook

- With a proprietary serum-free medium and through biological cloning, MedImmune has prepared a MDCK cell bank that is shown to be
  - Low in tumorigenicity and oncogenicity
  - High in virus productivity for both pandemic and seasonal influenza vaccine strains
- By identifying and optimizing critical process parameters MedImmune has developed a flu vaccine platform manufacture process that is
  - highly productive for seasonal (H1N1, H3N2 and B) and pandemic (H5N1, H7N3, H9N2) virus strains with yield >8 $\log_{10}$FFU/mL
  - Used to complete Phase I GMP clinical production campaigns successfully in spite of a last minute change in H1N1 virus
- Phase II Process Development is on-going with focuses on process scale up, robustness and shorter development time

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Posters #27 and #28

- Poster #27. Development of a Cell Culture Production Platform for Cold-Adapted Live Attenuated Influenza Vaccine (CAIV) Strains of FluMist®: Effects and Interactions of Medium Components, Trypsin, and Influenza Virus Family/Type in Process Productivity

- Poster #28. Development of a Cell Culture Production Platform for Cold-Adapted live attenuated Influenza Vaccine (CAIV) strains of FluMist®: Accelerated Development of a Fully Disposable Phase I Clinical Manufacturing Process