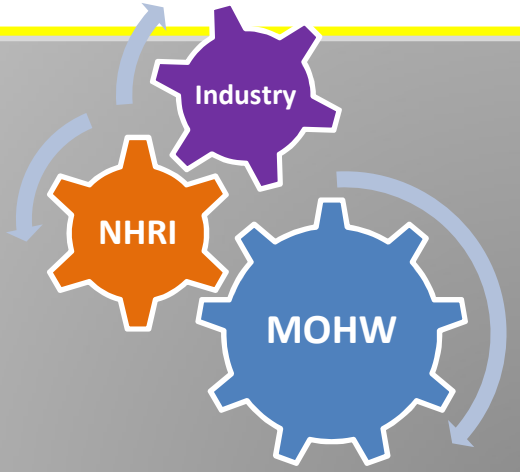
National Institute of Infectious Diseases and Vaccinology

Update of cell-based influenza pandemic vaccine development

Alan Yung-Chih Hu, PhD



Current Influenza (Flu) Vaccines on Market

Global market: US $4.4 billions in 2016

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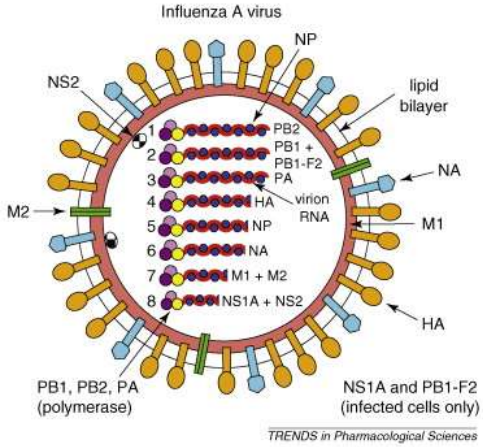
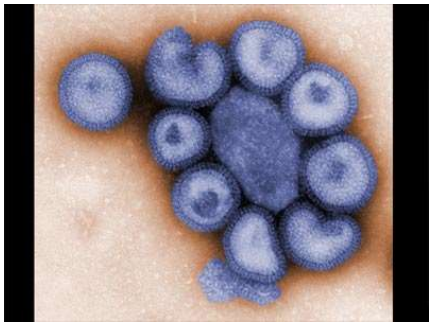


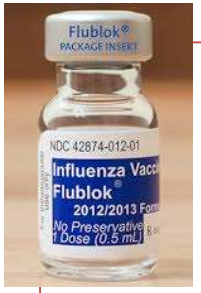


1945~

Egg-Based Flu Vaccines

2007 (Optaflu)

Cell-Based Flu Vaccines



2013 (FluBlok)

Recombinant Flu Vaccines

Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2019

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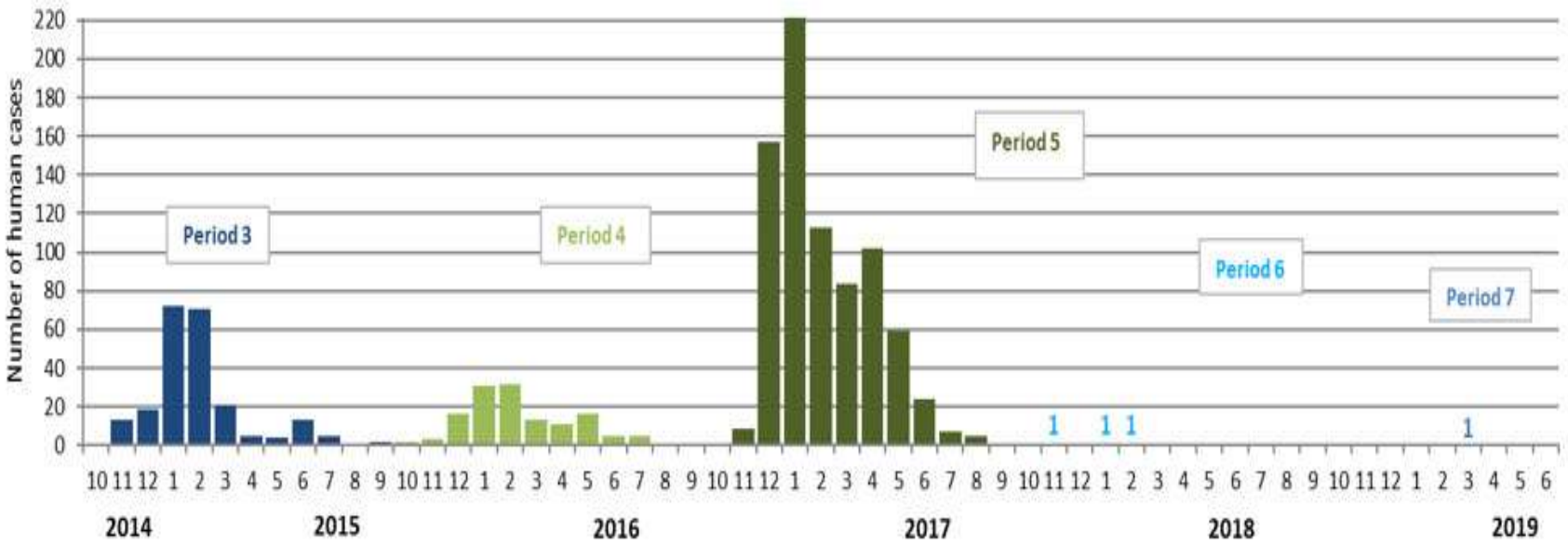
Country	2003-2009*		2010-2014**		2015		2016		2017		2018		2019		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	8	5	0	0	0	0	0	0	0	0	0	0	0	0	8	5
Bangladesh	1	0	6	1	1	0	0	0	0	0	0	0	0	0	8	1
Cambodia	9	7	47	30	0	0	0	0	0	0	0	0	0	0	56	37
Canada	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1
China	38	25	9	5	6	1	0	0	0	0	0	0	0	0	53	31
Djibouti	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Egypt	90	27	120	50	136	39	10	3	3	1	0	0	0	0	359	120
Indonesia	162	134	35	31	2	2	0	0	1	1	0	0	0	0	200	168
Iraq	3	2	0	0	0	0	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	2	2	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Myanmar	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Nepal	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
Nigeria	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Pakistan	3	1	0	0	0	0	0	0	0	0	0	0	0	0	3	1
Thailand	25	17	0	0	0	0	0	0	0	0	0	0	0	0	25	17
Turkey	12	4	0	0	0	0	0	0	0	0	0	0	0	0	12	4
Viet Nam	112	57	15	7	0	0	0	0	0	0	0	0	0	0	127	64
Total	**468**	**282**	**233**	**125**	**145**	**42**	**10**	**3**	**4**	**2**	**0**	**0**	**1**	**1**	**861**	**455**

* 2003-2009 total figures. Breakdowns by year available on subsequent tables.
** 2010-2014 total figures. Breakdowns by year available on subsequent tables.
Total number of cases includes number of deaths.
WHO reports only laboratory cases.
All dates refer to onset of illness.
Source: WHO/GIP, data in HQ as of 24 June 2019

World Health Organization

Incidence of officially reported human cases by month, based on onset date from October 2014 (beginning of period 3) to 03 July 2019



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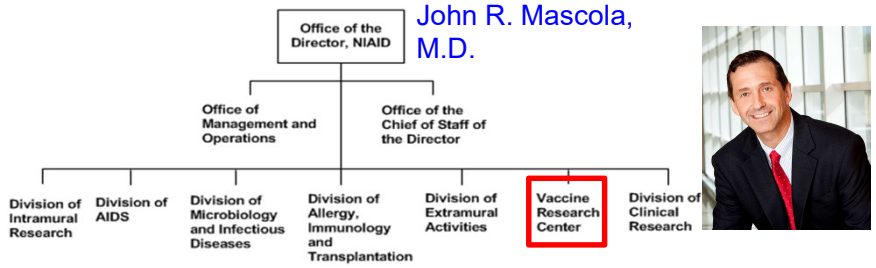
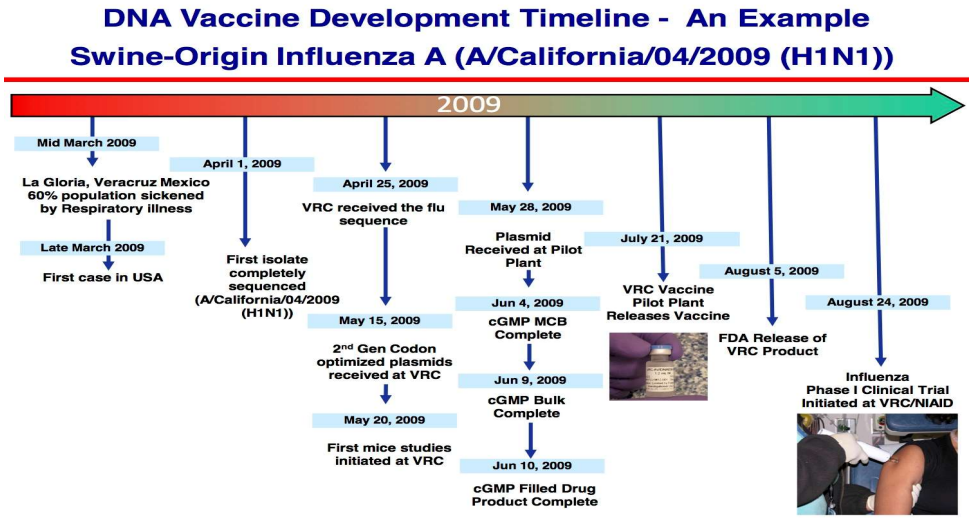
National Institute of Allergy and Infectious Diseases (NIAID)



John R. Mascola, M.D.

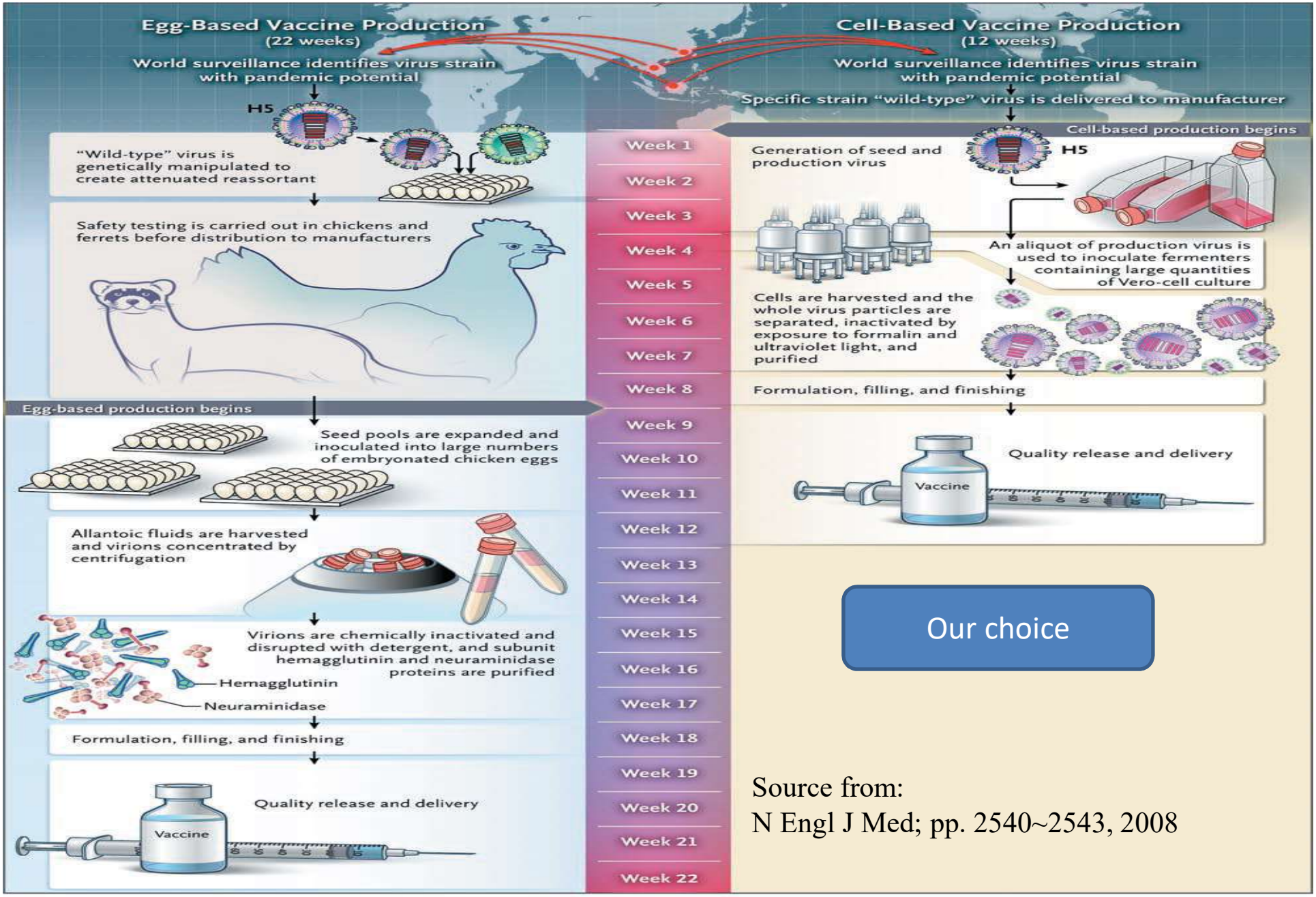
Dr. David Lindsay

Dr. KC Cheng



Zika DNA vaccine development: from discovery to FDA release for phase I trial: 61 days

- Site visit of four production trains
- The update of single-use concept
- Good understand of VRC mission to national need
- Initializing collaboration of HEK-based VLP platform



Our choice

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

Key elements for cell-based vaccine development

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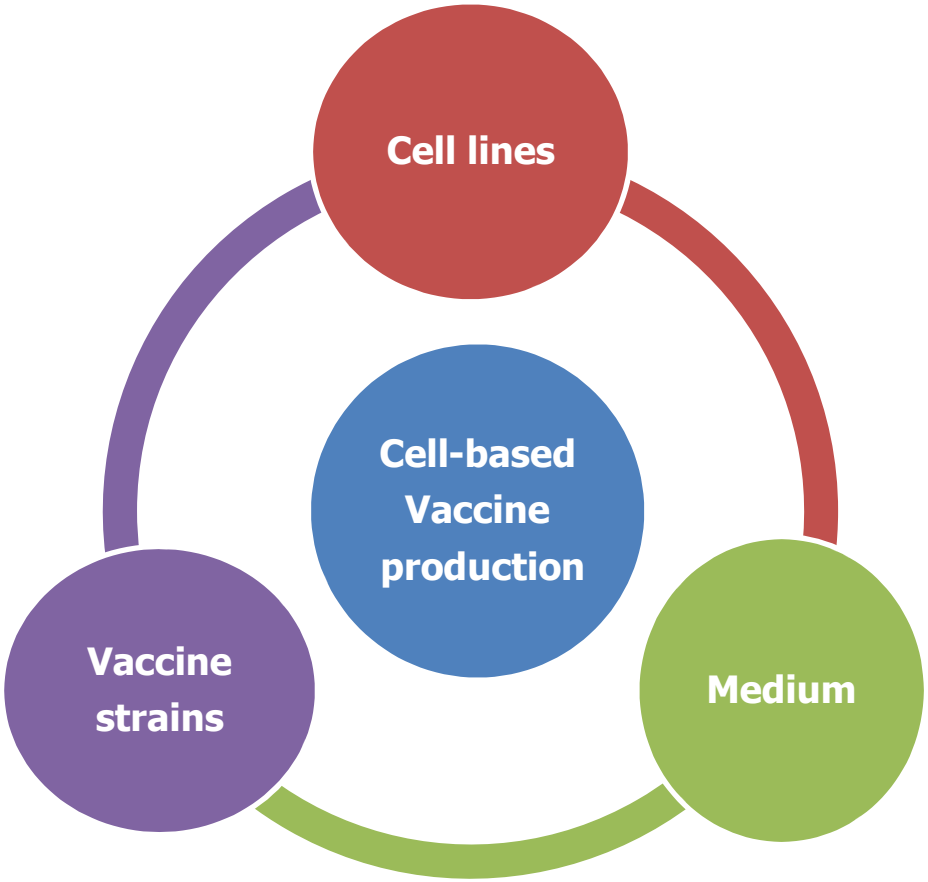


Upstream process

- Single use bioreactor
- Scaling-up strategy

Downstream process

- TFF
- Chromatography

Development history - Process development of influenza vaccine production

Year 2005-2010

1st generation

- Roller bottle technology
- Ultracentrifugation
- benzonase addition

H5N1 phase I trial completion

Year 2013-2015

2nd generation

- Bioreactor with microcarrier technology
- Liquid Chromatography

H7N9 phase I/II trial completion

Year 2015~

3rd generation

- Suspension sMDCK technology
- Improved downstream purification

High potential for commercial market

Journal of Microbiology, Immunology and Infection (2013) 46, 448–455

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-jmii.com

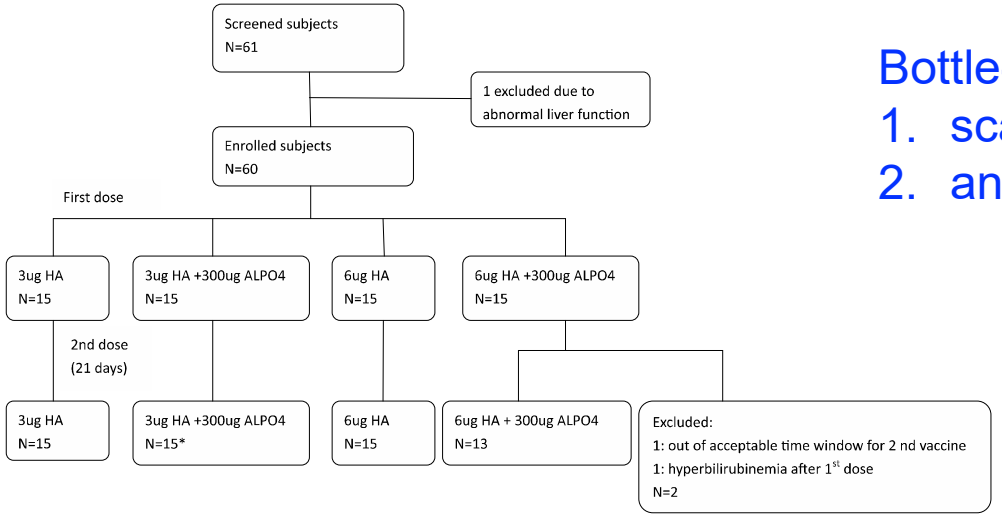
ORIGINAL ARTICLE

The Madin-Darby canine kidney cell culture derived influenza A/H5N1 vaccine: A Phase I trial in Taiwan

Sung-Ching Pan [a,b], Hsiang-Chi Kung [a], Tsui-Mai Kao [a], Hsio Wu [a], Shao-Xing Dong [a], Mei-Hua Hu [c], Ai-Hsiang Chou [c], Pele Chong [c,d], Szu-Min Hsieh [a,b,*], Shan-Chwen Chang [a,b]

[a] Department of Internal Medicine, National Taiwan University Hospital, Taiwan
[b] College of Medicine, National Taiwan University, Taipei, Taiwan
[c] Vaccine R&D Center, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Zhunan Town, Miaoli County, Taiwan
[d] Graduate Institute of Immunology, China Medical University, Taichung, Taiwan

Received 21 June 2011; received in revised form 20 July 2012; accepted 30 July 2012



Bottle-neck issues:
1. scaling-up
2. antigen quality

Figure 1. Enrollment and follow up of the study participants. *One participant was excluded from immunogenicity analysis, even though they received two doses of vaccination, due to receiving another investigational vaccine.

	Group 1 15μ HA	Group 2 15μ HA+ Al(OH)$_3$	Group 3 30μ HA	Group 4 30μ HA+ Al(OH)$_3$
SPR	42.2%	39.6%	51.0%	64.6%

Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Safety and immunogenicity of an inactivated cell culture-derived H7N9 influenza vaccine in healthy adults: A phase I/II, prospective, randomized, open-label trial

Un-In Wu[a,1], Szu-Min Hsieh[a,1], Wen-Sen Lee[b,c], Ning-Chi Wang[d], Hsiang-Chi Kung[a], Tsong-Yih Ou[b], Fu-Lun Chen[b], Te-Yu Lin[d], Yee-Chun Chen[a,e,f], Shan-Chwen Chang[a,e,*]

[a] Division of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
[b] Division of Infectious Diseases, Department of Internal Medicine, Taipei Municipal Wan-Fang Hospital, Taipei, Taiwan
[c] Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[d] Division of Infectious Diseases, Department of Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan
[e] Department of Medicine, National Taiwan University College of Medicine, Taipei, Taiwan
[f] National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan

Table 2

Summary of GMT, seroconversion rates, seroconversion factors, and seroprotection rates for serum anti-HA antibody titers.

		n	Group 1 15 μg HA	n	Group 2 15 μg HA + Al(OH)$_3$	n	Group 3 30 μg HA	n	Group 4 30 μg HA + Al(OH)$_3$
GMT[a]	Day 1	45	6.1 (5.4, 6.8)	49	6.2 (5.5, 7.0)	50	5.9 (5.3, 6.6)	49	6.4 (5.6, 7.3)
	Day 22	45	11.7 (9.3, 14.7)	49	9.4 (7.9, 11.3)	50	15.2 (11.9, 19.2)	49	12.4 (9.6, 15.9)
	Day 43	45	24.1 (19.1, 30.4)	48	21.8 (17.7, 26.8)	49	32.8 (25.9, 41.6)	48	36.2 (28.5, 45.9)
SCR	Day 22	45	11.1% (3.7%, 24.1%)	49	4.1% (0.5%, 14.0%)	50	20.0% (10.0%, 33.7%)	49	10.2% (3.4%, 22.2%)
	Day 43	45	40.0% (25.7%, 55.7%)	48	37.5% (24.0%, 52.6%)	49	46.9% (32.5%, 61.7%)	48	64.6% (49.5%, 77.8%)
SCF	Day 22	45	1.9 (1.5, 2.4)	49	1.5 (1.3, 1.8)	50	2.6 (2.0, 3.3)	49	1.9 (1.5, 2.5)
	Day 43	45	3.9 (3.1, 5.0)	48	3.6 (2.9, 4.4)	49	5.5 (4.2, 7.3)	48	5.7 (4.4, 7.4)
SPR	Day 22	45	11.1% (3.7%, 24.1%)	49	6.1% (1.3%, 16.9%)	50	22.0% (11.5%, 36.0%)	49	12.2% (4.6%, 24.8%)
	Day 43	45	42.2% (27.7%, 57.8%)	48	39.6% (25.8%, 54.7%)	49	51.0% (36.3%, 65.6%)	48	64.6% (49.5%, 77.8%)

Data are expressed as value (2-sided 95% CI).

GMT: geometric mean titer; HA: hemagglutinin; SCR: seroconversion rate; SCF: seroconversion factor; SPR: seroprotection rate.

[a] GMT were compared among the groups using one-way ANOVA. *P* value = 0.852 (day 1); 0.031 (day 22); 0.004 (day 43).

Is it possible to develop suspension MDCK cells?

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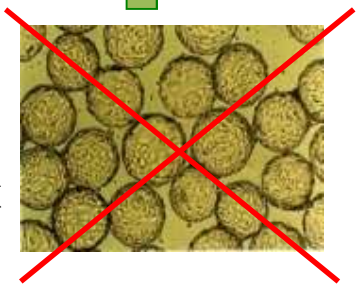

- 製程複雜且較耗人力
- 消化酶作用機制較難控制
- 製程時間較長

Collaboration with Irvine Scientific (US)

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OPEN ACCESS Freely available online

2011 PLoS one

Production of Inactivated Influenza H5N1 Vaccines from MDCK Cells in Serum-Free Medium

Alan Yung-Chih Hu[1], Yu-Fen Tseng[1], Tsai-Chuan Weng[1], Chien-Chun Liao[1], Johnson Wu[1], Ai-Hsiang Chou[1], Hsin-Ju Chao[1], Anna Gu[1], Janice Chen[1], Su-Chen Lin[1], Chia-Hsin Hsiao[1], Suh-Chin Wu[1,2], Pele Chong[1,3]*

1 Vaccine Research and Development Center, National Health Research Institutes, Zhunan, Taiwan Authority, 2 Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan Authority, 3 Graduate Institute of Immunology, China Medical University, Taichung, Taiwan Authority

Cell_based Inactivated Influenza Vaccine Production by MDCK Cells Cultured in Chemically-Defined Medium

Alan Yung-Chih Hu[1], Tsai-Chuan Weng[1], Jenny Bang[2], Jessie H.-T. Ni[2]

[1]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taiwan
[2]Department of Research and Development, Irvine Scientific, Santa Ana, California

Tel: +886 988219928, Fax: +886 37583009, alanhu@nhri.org.tw

Introduction

Influenza is a highly contagious disease that usually results in fever and respiratory symptoms and some severe cases can lead to hospitalization and death. In response to rapid antigenic drift in influenza viruses, vaccination is considered as the most effective intervention. Traditionally these vaccines are produced in chicken embryonated eggs. However, in the case of a pandemic outbreak, this egg-based production system may not be quickly enough to meet the surge demand. The limitations associated with egg-based vaccines are reliable egg supplies, prolonged cultivation periods, and cumbersome operations and these limitations resulted in spurred exploration of alternatives. MDCK cells are widely being considered as an alternative host to embryonated eggs for influenza virus propagation.

Cell culture medium without serum supplement is the primary goal to improve product safety because most of vaccines are used for healthy human. Although the current availability of hydrolysate-supplemented serum-free media greatly reduced worries related to the serum addition which concerns many risks associated with lot-to-lot variations, the undefined components are still remaining safety concerns. In this study, we present the successful adaption of influenza H5N1/H7N9 vaccine production from Hydrolysate containing media (Optipro, Invitrogen) to Chemically-defined media (BalanCD, Irvine Scientific) in a small-scale microcarrier cell culture system. The purpose of this study was to evaluate the feasibility of chemically-defined medium as a critical process parameter for developing large-scale production (illustrated on the lower right of this poster). The results of this study provided a valuable information for the future work of process development.

Materials and Methods

WHO candidate vaccine H5N1 (NIBRG-14) and H7N9 (NIBRG-268) strains obtained from the NIBSC, U.K. were grew in adherent MDCK cells. The serum-free medium was Optipro (Invitrogen, US); and chemically-define medium was BalanCD (Irvine Scientific, US). The microcarriers (Cytodex 1, GE Healthcare) were hydrated in PBS according to the manufacturers' instructions. When the cells were grown confluent on microcarriers in 250-ml spinner flasks (Corning, US). The H5N1 and H7N9 viruses were separately added with low MOI for virus replication. Cell counting was carried out by the trypan blue method. Virus titers were determined by TCID50 assay and hemagglutination (HA) assay.

Results

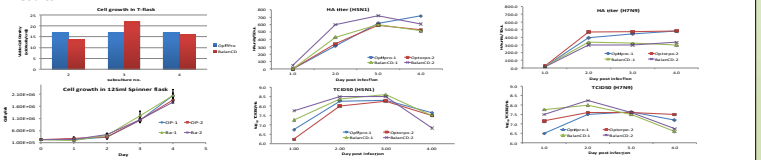

Figure 1. MDCK cell growth in static (T-flask) and dynamic (microcarriers) cultures.

Figure 2. Viral titers of H5N1 vaccine strain cultured in MDCK cells.

Figure 3. Viral titers of H7N9 vaccine strain cultured in MDCK cells.

Conclusions

BalanCD media were evaluated in both static and microcarrier cultures for the production of H5N1 and H7N9 viral vaccines using MDCK cells. In both cases, we achieved comparable cell growth and virus yield to a commercial hydrolysate containing media (Optipro, Invitrogen). The data presented in this study demonstrate that BalanCD media is beneficial alternative to cell-based vaccine-manufacturing processes that not only provide higher quality of raw materials for stringent regulatory requirements but also lower cost of media shipment with powder-form formulation.

Acknowledgments

The authors thank the UK NIBSC for supplying the H5N1 (NIBRG-14) & H7N9 (NIBRG-268) candidate vaccine strains, Irvine Scientific for medium supply, and NHRI and MOHW for financial support.

Attached cells:
BalanCD MDCK, BalanCD Vero

2013
USA

2015
Spain

The development of sMDCK cells -- 3rd generation

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aMDCK Bank (P62)
DMEM+5%FBS

Direct adapted into SFM

Attached on Cytodex1 microcarriers

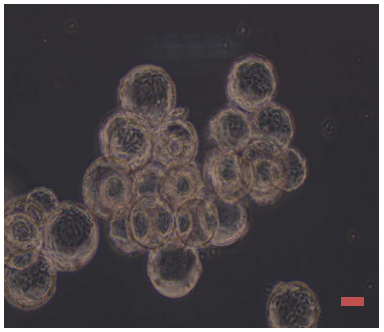
Virus Productivity of aMDCK

	HA (units/ 100ul)	log_{10} $TCID_{50}$/ml
H7N9 (RG268)	574.0	7.6
H5N1 (RG14)	612.7	8.6

Virus Productivity of sMDCK

	HA (units/ 100ul)	log_{10} $TCID_{50}$/ml
H7N9 (RG268)	996.3	7.9
H5N1 (RG14)	989.0	8.6

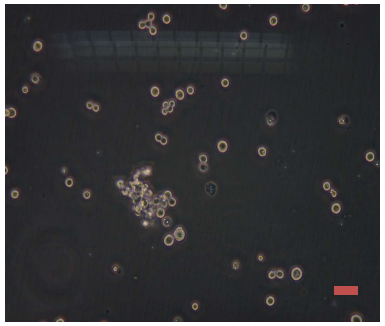
Attached MDCK Bank (P62)
(DMEM+5%FBS)



50μm

1. Attached on Cytodex1 as suspension culture
2. Culture Medium: SFM
3. Doubling time: 30~35 hours

Suspension MDCK Bank (P70)
(M1+M2+M3)



50 μm

1. Free suspension culture
2. Culture Medium: M1 + M2 +M3
3. Doubling time: 30~35 hours

Serial adapted into M1 Medium + <0.5% FBS

A-MDCK adapted to free suspension culture

sMDCK Bank (P67)
Freeze medium

Free suspension culture

Serial adapted into M1+M2+M3

sMDCK Bank (P70)
M1+M2+M3

BalanCD simple MDCK medium from Fujifilm Irvine Scientific

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A suitable cell culture medium for a cell-based vaccine manufacturing process is critical because it can significantly affect the overall efficiency, consistency of production, and reduces contamination risks and potential inhibitors.

BalanCD® simple MDCK is commercially-available animal component-free and chemically-defined MDCK medium that can be used towards building a robust, cost-effective, and regulatory-friendly mammalian cell-based vaccine manufacturing platform.

Two manufacturing sites:
1. California, US 2. Tokyo, Japan

FUJIFILM
Value from Innovation

IrvineScientific

Fujifilm completes acquisition of Irvine Scientific Sales Company and IS Japan, leading companies of cell culture media

FUJIFILM Corporation (President: Kenji Sukeno) announced today that it has completed the acquisition of Irvine Scientific Sales Company, Inc. (ISUS) and IS JAPAN CO.,LTD. (ISJ), leading companies in cell culture media for about US$800 millions Jun 04, 2018

Tumorigenicity in mice

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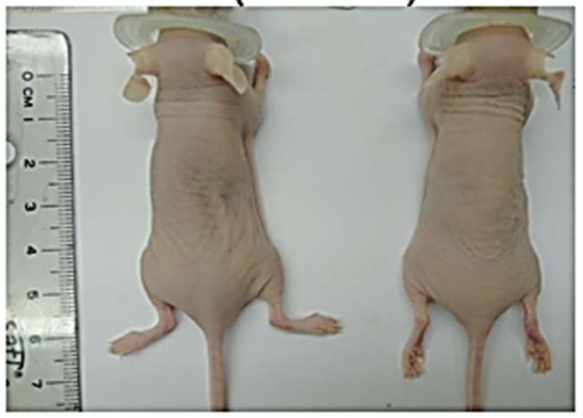
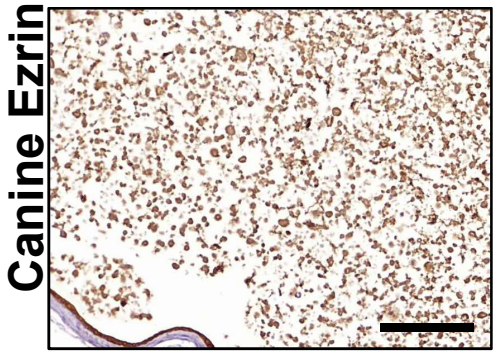
- **FDA guidance for industry:**
  - Test animals: BALB/c nude female mice
  - Age: 8-9 weeks old
  - Test cells:
    - ✓ Positive control cells (Hela cells)
    - ✓ Exp cells (No.1-4)
  - Cell number: $1x10^7$/1XPBS
  - Injection site: between the scapulae

Hela cells

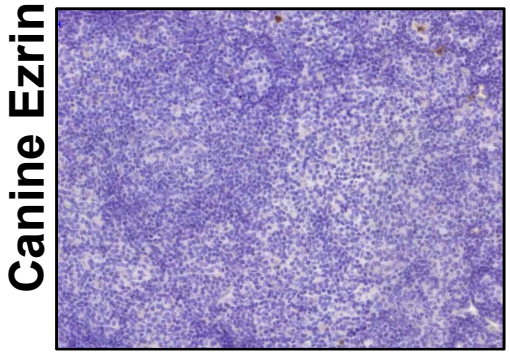
NO.3 cells (sMDCK)



Subcutaneous inoculation



Lymph node



H5N1 flu virus production test (3rd ~10th passages)

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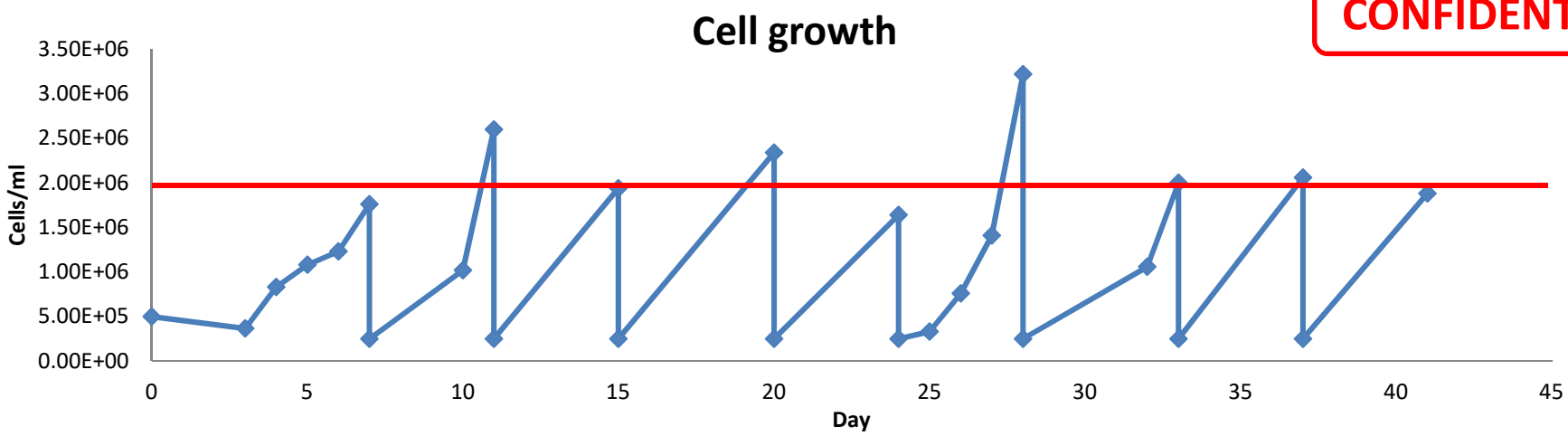

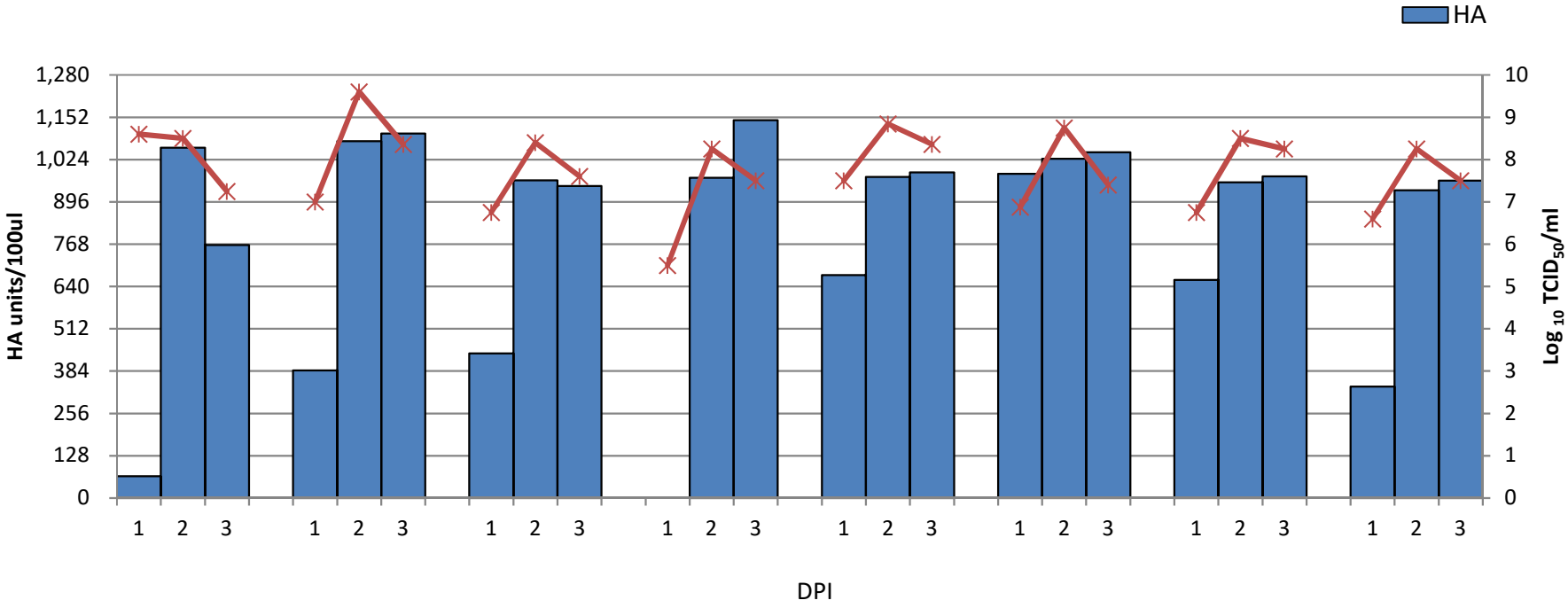

H7N9 flu virus production test (3rd ~10th passages)

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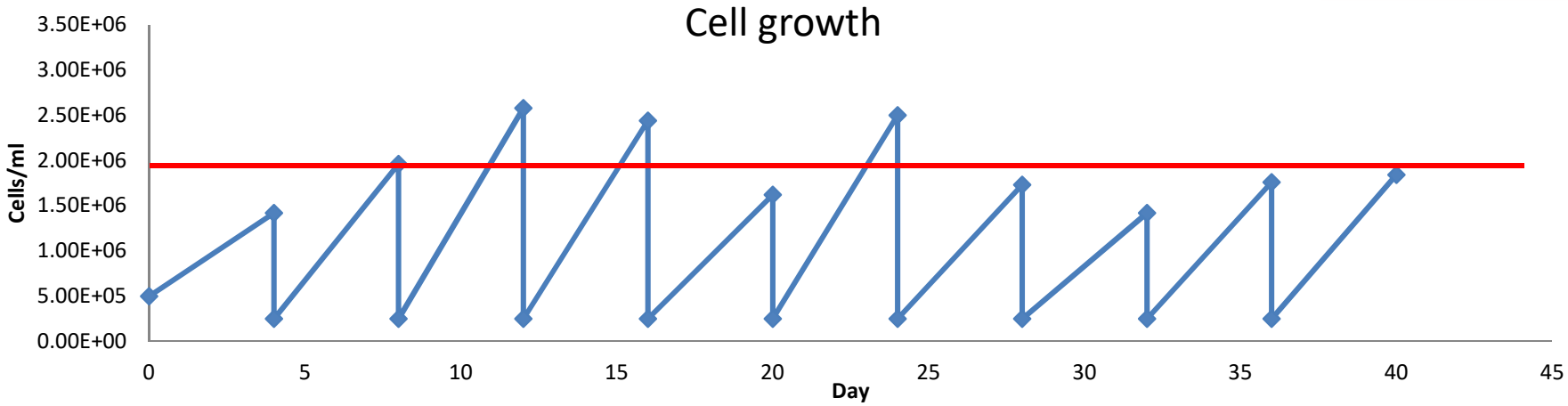

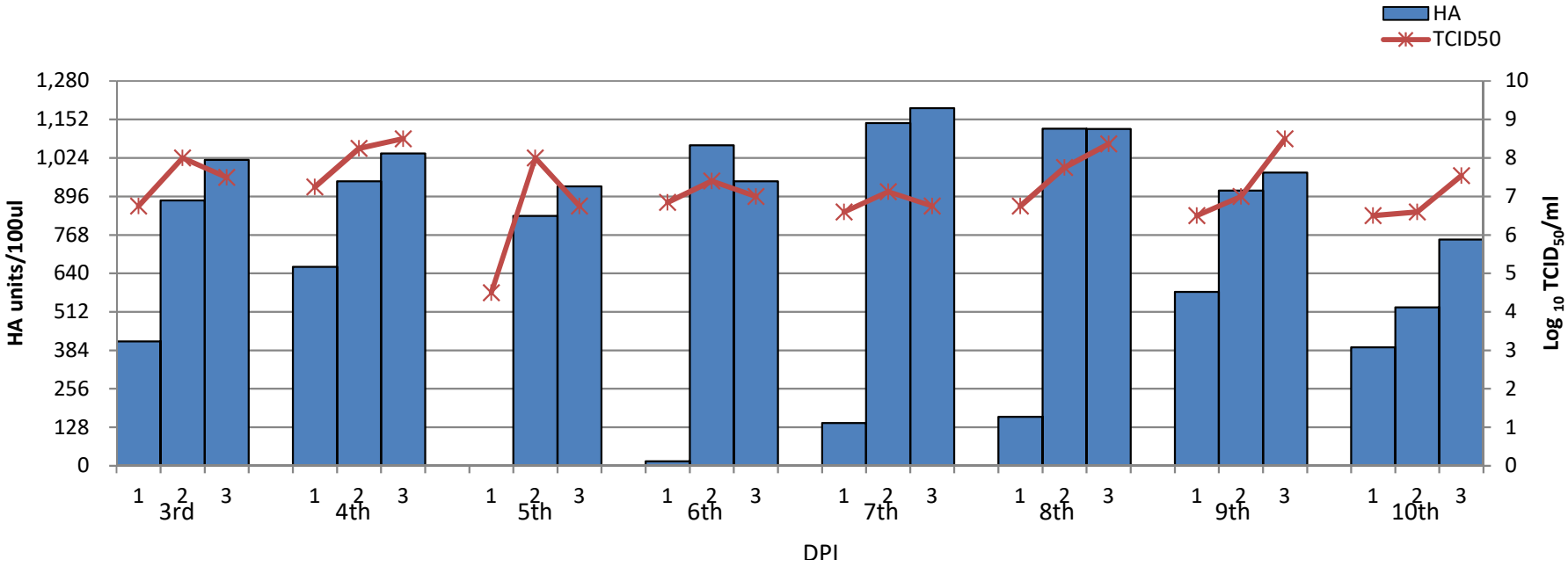

Bioreactor run with cell retention on H7N9 virus

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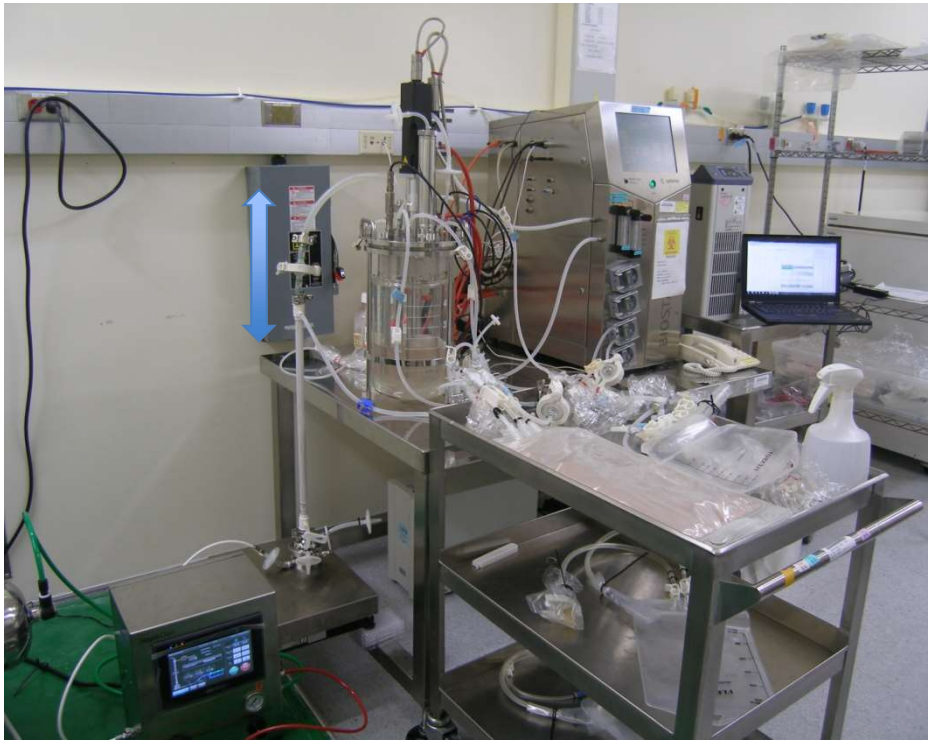

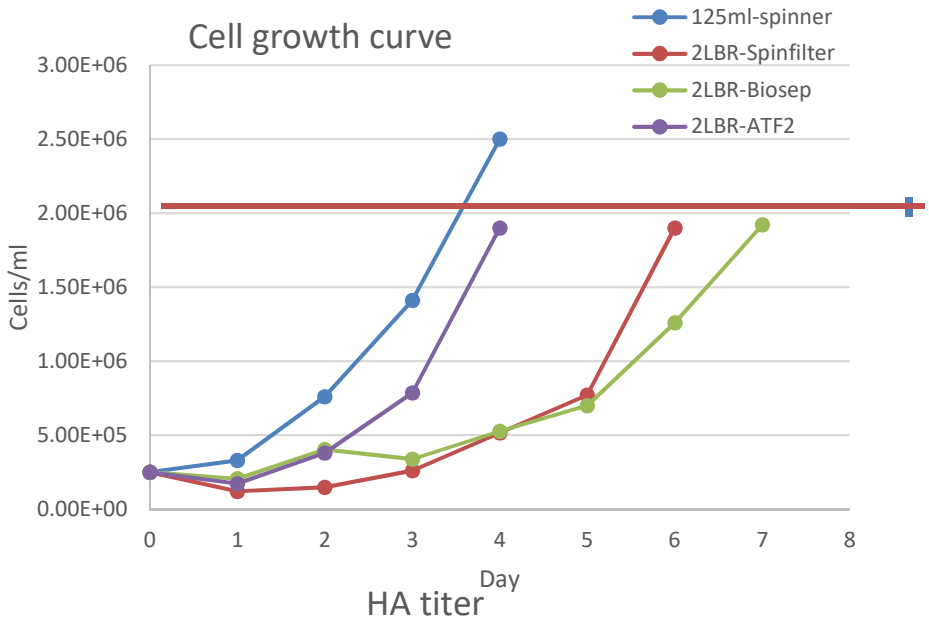

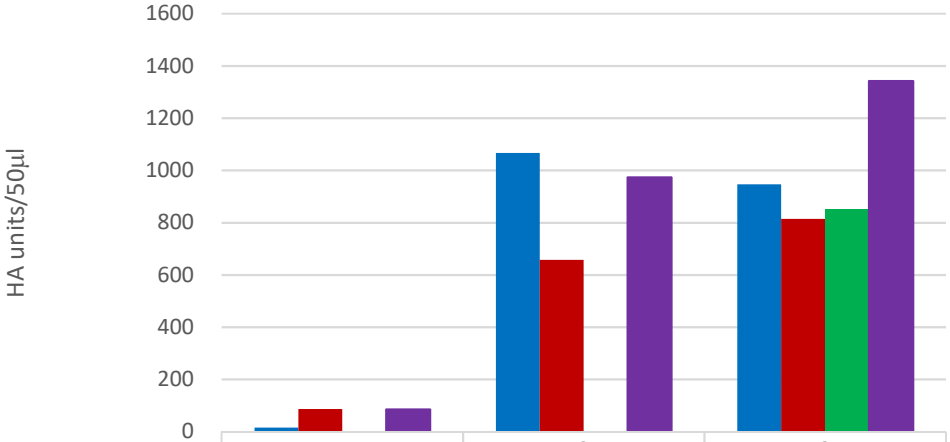

	1	2	3
HA 125ml-spinner	15.1	1066.5	946.2
HA 2LBR-Spinfilter	85.5	657.2	814.7
HA 2LBR-Biosep			853.2
HA 2LBR-ATF2	85.3	973.5	1342.4

Upstream process development of 50L single-use bioreactor

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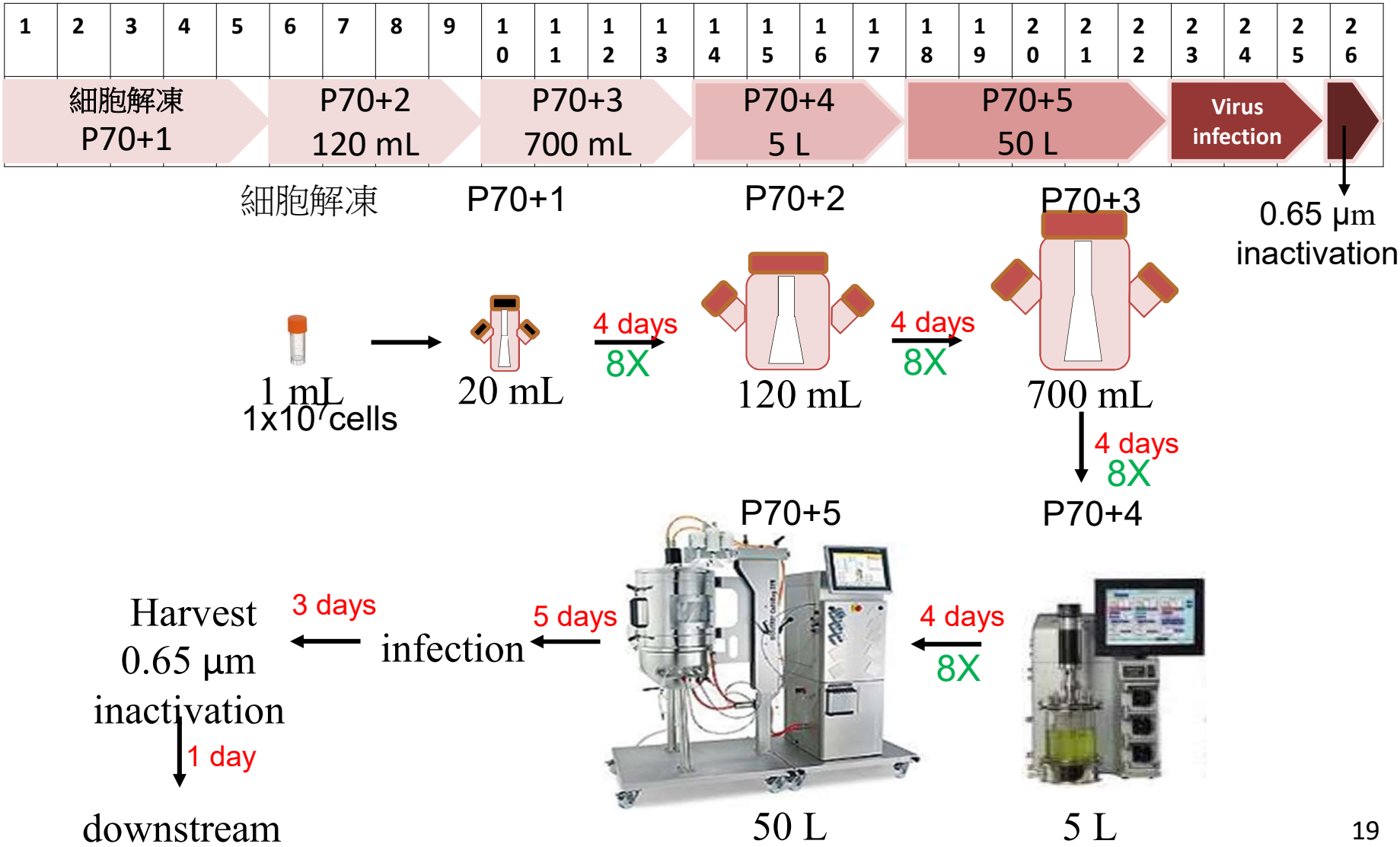

50 L bioreactor run

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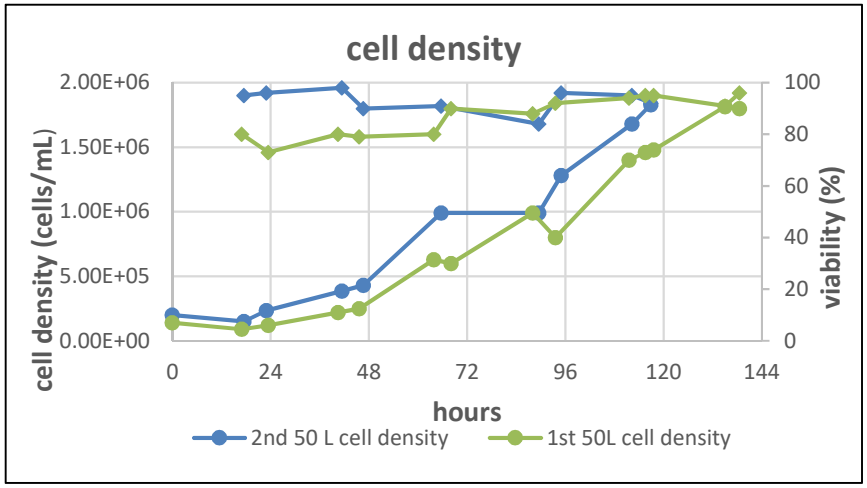

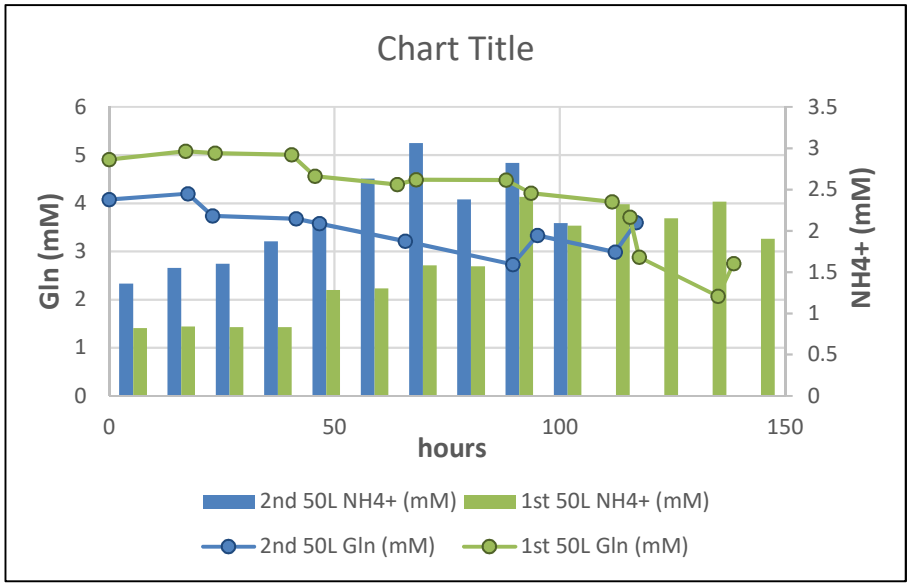

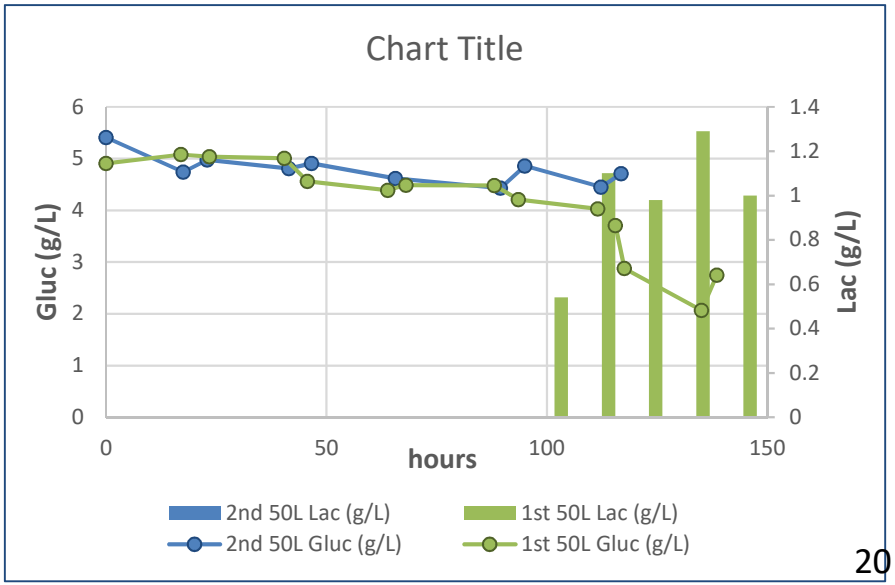

Vaccine xxx (xxxx) xxx-xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com

The stability and immunogenicity of inactivated MDCK cell-derived influenza H7N9 viruses

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Inactivated influenza vaccine
Stability
Suspension MDCK
H7N9

ABSTRACT

In recent years, cell-based influenza vaccines have gained a great interest over the egg-based vaccines. Several inactivated H7N9 vaccines have been evaluated in clinical trials, including whole-virion vaccines, split vaccines and subunit vaccines. Recently, we developed a new suspension MDCK (sMDCK) cell line for influenza viruses production. However, the properties of purified antigen from sMDCK cells remain unclear. In this study, the stability of influenza H7N9 vaccine bulk derived from sMDCK cells was investigated, and the data were compared with the vaccine antigen derived from our characterized adhesion MDCK (aMDCK) cells in serum-free medium. The influenza H7N9 bulks derived from sMDCK and aMDCK cells were stored at 2– 8 °C for different periods of time, and a number of parameters selected to monitor the H7N9 vaccine antigen stability were evaluated at each interval (1, 3 and 12 months). The monitored parameters included virus morphology, hemagglutinin (HA) activity, HA concentration, antigenicity, and immunogenicity. The sMDCK-derived H7N9 bulk showed similar morphology to that of the aMDCK-derived H7N9 bulk, and there were no obvious changes after the extended storage periods. Furthermore, the HA titer, HA concentration, and antigenicity of sMDCK-derived H7N9 bulk were stable after 28 months of storage. Finally, the results of hemagglutination inhibition and neutralization tests showed that sMDCK- and aMDCK-derived H7N9 vaccines had comparable immunogenicity. These results indicated that sMDCK-derived H7N9 bulk has good stability compared to that of aMDCK-derived H7N9 bulk. Thus, the newly developed suspension MDCK cell line shows a great alternative for manufacturing cell-based influenza vaccines.

Fig. 1. Test schedule for the stability program of H7N9 bulks derived from different manufacturing platforms. sMDCK- and aMDCK-derived H7N9 bulks were stored at 2 8 °C for different periods of time, and a number of parameters were measured to monitor the H7N9 vaccine antigen stability at different periods (1st, 3rd and 12th month). These monitoring parameters included virus morphology, HA titer, HA concentration, antigenicity, and immunogenicity.

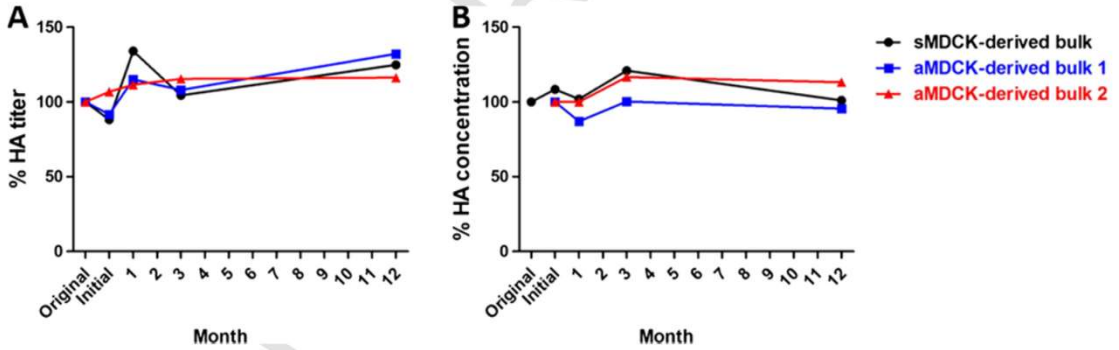

Fig. 3. HA titer and HA concentration of H7N9 bulks from different platforms during the stability study. sMDCK- and aMDCK-derived H7N9 bulks were stored at 2 8 °C, and their HA titer (A) and HA concentration (B) were measured at the indicated time points by HA and SRID assays, respectively. The relative HA titer and HA concentration were expressed as a percentage relative to the original value or the value detected at the initial time point of the stability program.

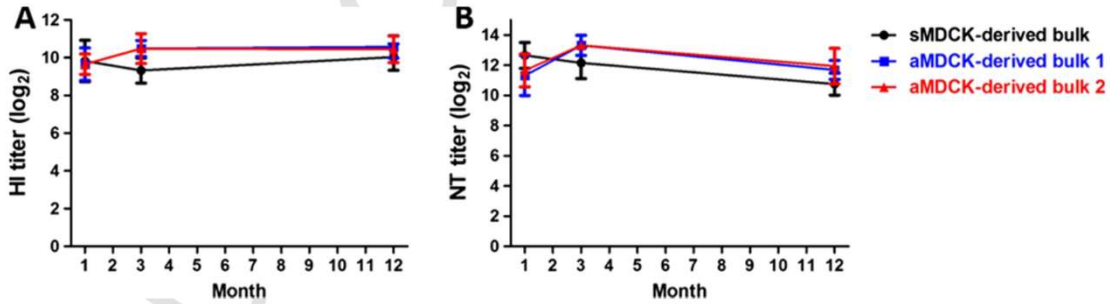

Fig. 4. Immunogenic analysis of various H7N9 bulks during the stability study. sMDCK- and aMDCK-derived H7N9 bulks were stored at 2 8 °C. 0.2 g of the HA antigen dose of MDCK-derived H7N9 bulks from the indicated time points were mixed with $Al(OH)_3$ adjuvant, and administered in BALB/c mice (n=6 per group) intramuscularly at day 0 and day 14. The immunogenicity of various bulks was confirmed by HI and NT assays, using the serum collected at day 28. Error bars represent the 95% confidence interval.

TEM images of H7N9 bulks from sMDCK- and aMDCK-derived cells

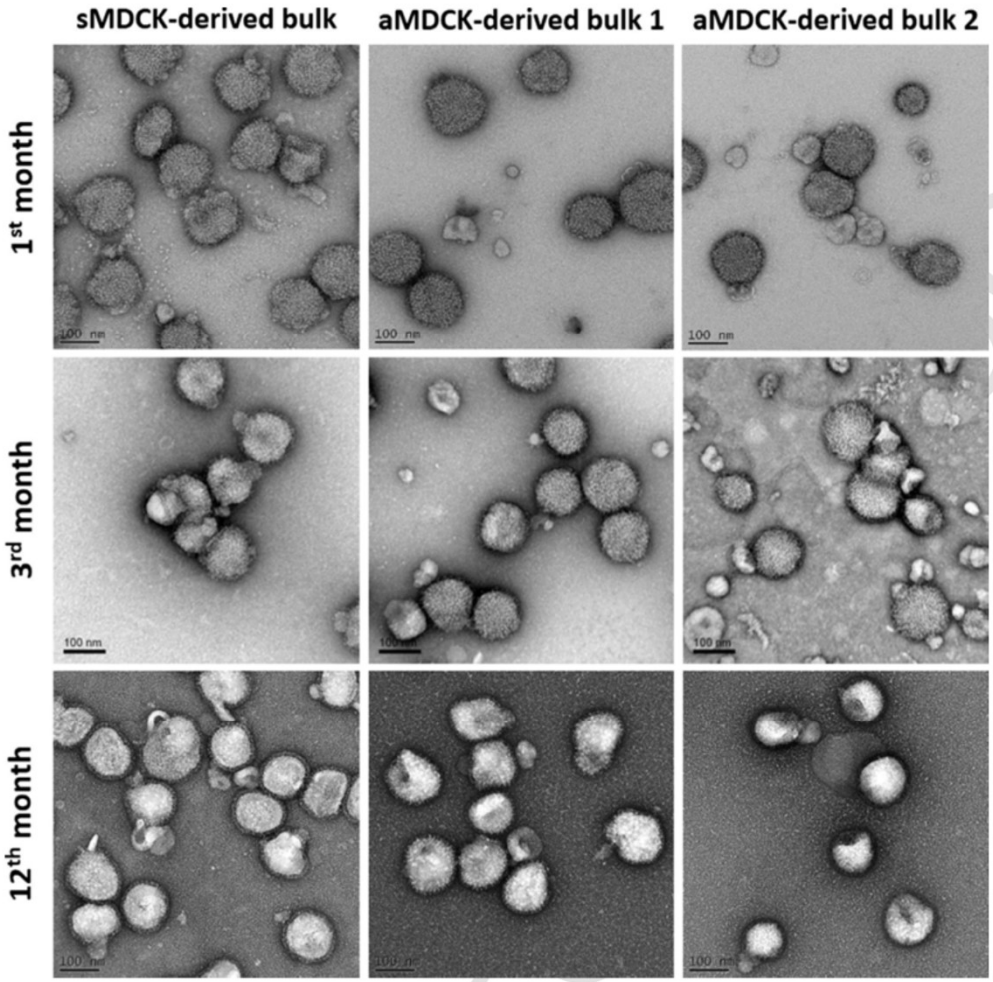

Fig. 2. EM images of H7N9 bulks from different platforms. sMDCK- and aMDCK-derived H7N9 bulks were stored at 2 8 °C, and the morphology of the H7N9 viral particles was analyzed at the 1st, 3rd and 12th month of the stability program. Viral particles were negatively stained with 2% uranyl acetate and the images were captured using electron microscopy.

Flowchart of generation candidate vaccine viruses

協助單位

step1	• Collection of specimens and disease/epidemiological data	農委會 疾管署
step2	• Diagnosis, virus isolation in MDCK, primary analysis	
step3	• Ferret antisera production	預醫所
step4	• Thorough antigenic and genetic analysis	
step5	• Review and selection of candidate viruses for vaccine use	
step6	• Reassortment of high-growth viruses using reverse genetics (full safety testing)	預醫所 家衛所
step7	• Evaluation of growth property	
step8	• Development of standardized reagents for inactivated vaccines	
step9	• Antigenic and genetic characterization of reassortants	

Vaccine production

Timeline for the generation of candidate vaccine viruses by reverse genetic technology

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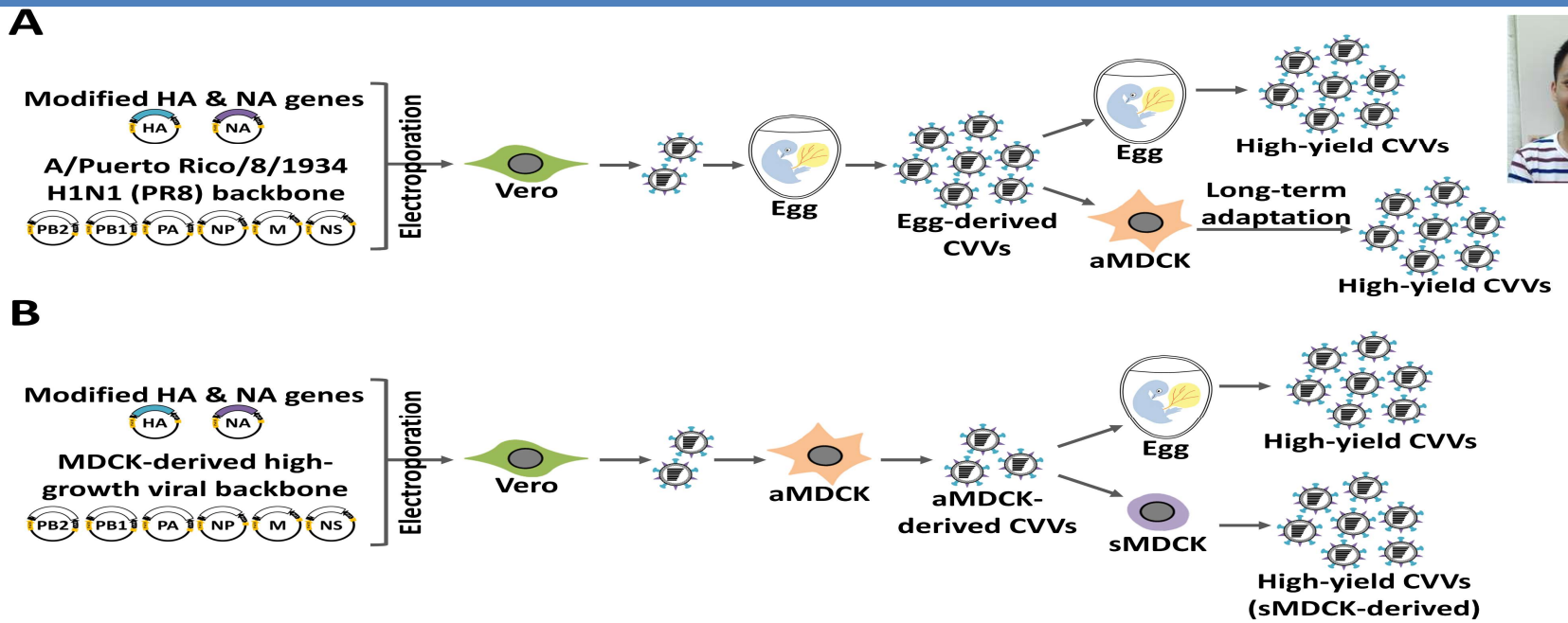

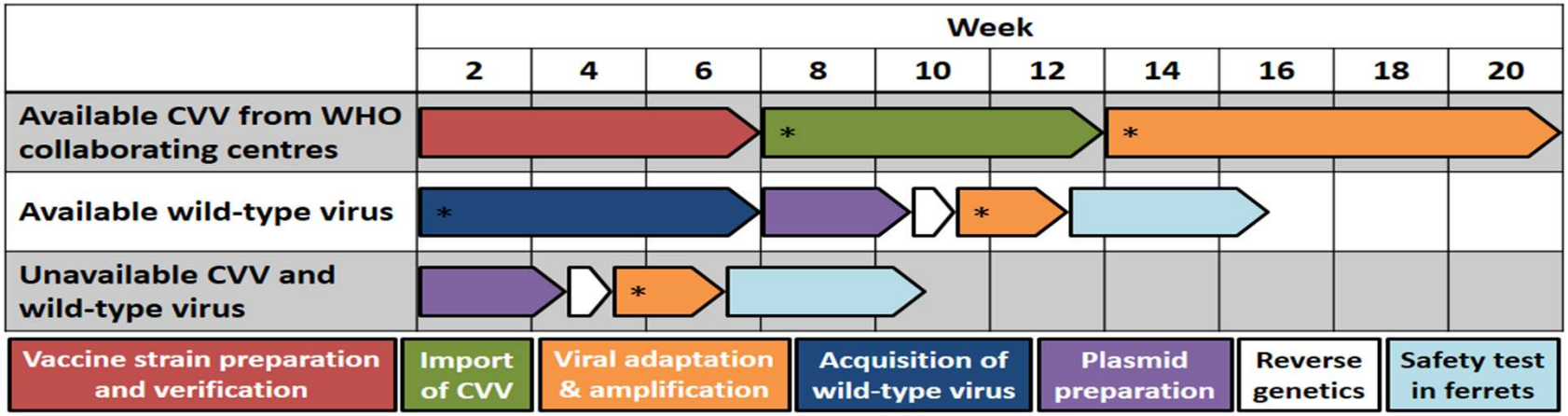

Viral titers of H7N9 CVVs after serial passaging in Vero cells, aMDCK cells, and chicken embryonic eggs.

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	HA titer (HAU/50 µL) at each passage						TCID$_{50}$ (Virions/ml)	
CVVs	V1*	V1aM1*	V1aM2*	V1aM3*	V1aM3E1 *		V1aM3*	V1aM3E1 *
NHRI-RG3	4	64	64	256	2048		$10^{7.30}$	$10^{7.04}$
NHRI-RG4	8	64	64	256	2048		$10^{7.40}$	$10^{7.04}$
NHRI-RG5	64	256	256	256	2048		$10^{7.51}$	$10^{7.80}$
NHRI-RG6	64	128	256	256	2048		$10^{7.77}$	$10^{7.04}$

Candidate vaccine virus preparation using synthetic HA& NA plasmids and reversed genetics

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Spent one year still could not received H5N6 wide type virus from the Council of Agriculture

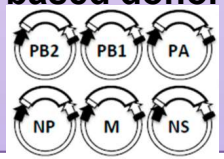
Plasmids preparation

H5 and H7 highly pathogenic avian influenza viruses (RG3 viruses)

Example: Modified HA gene of H5N6 virus (A/duck/Taiwan/1702004/2017)

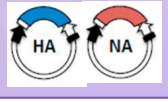
Highly pathogenic	Low pathogenic
Polybasic cleavage site; RNSPLRERRRKRGLF	Monobasic cleavage site; RNSPLGETRGLF

MDCK- or Vero-based donor



+

Modified HA & NA



Reverse genetics and Viral amplification

Vero cells
↓
MDCK_Passage 1 (6 well plate)
↓
MDCK_Passage 2 (T25)
↓
MDCK_Passage 3 (T150 for bank)

Characterization/Verification

Candidate vaccine virus

- Growth property (HA & $TCID_{50}$ titer)
- Antigenicity (HI titer)
- Immunogenicity
- Genetic stability (gene sequence)
- Biosafety
  - Pathogenicity in ferret & egg embryo
  - Plaque-forming ability without trypsin

↓

Candidate vaccine strain

Viral strain	H5N1	1st H7N9	H5N6 NHRI-RG1	A/Guangdong/17SF003/2016 NHRI-RG3	A/Hong Kong/125/2017 NHRIR-G4	A/Guangdong/SP440/2016 NHRI-RG5	A/Taiwan/1/2017 NHRI-RG6
aMDCK	612	574	689	128	256	256	128
sMDCK	**989**	**996**	**1409**	**1024**	**1024**	**1024**	**1024**

Growth properties of reassortant H5N6 and H7N9 viruses in aMDCK and sMDCK cells

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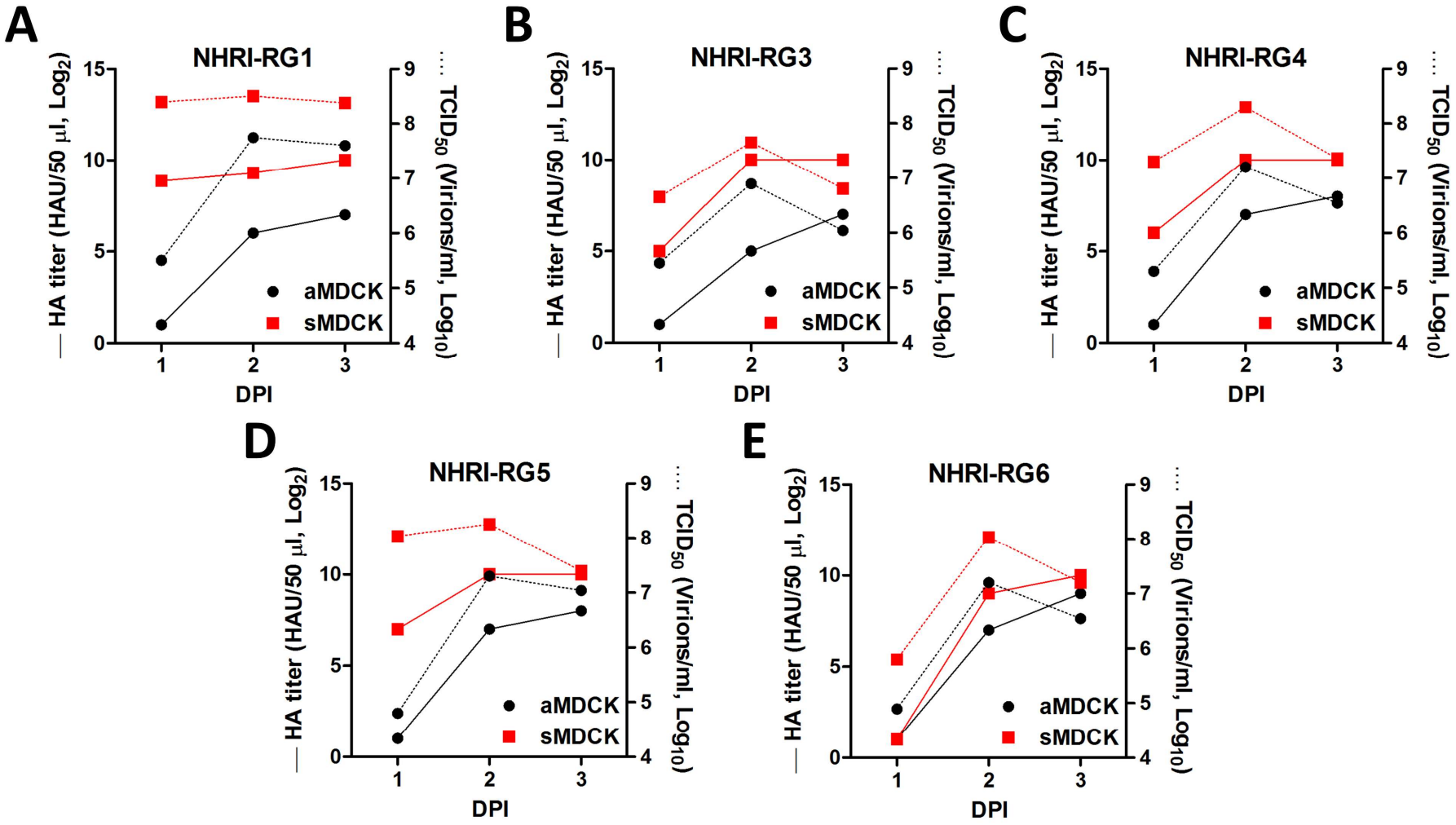

HI activity of mouse serum against the 1st and 5th wave H7N9 viruses

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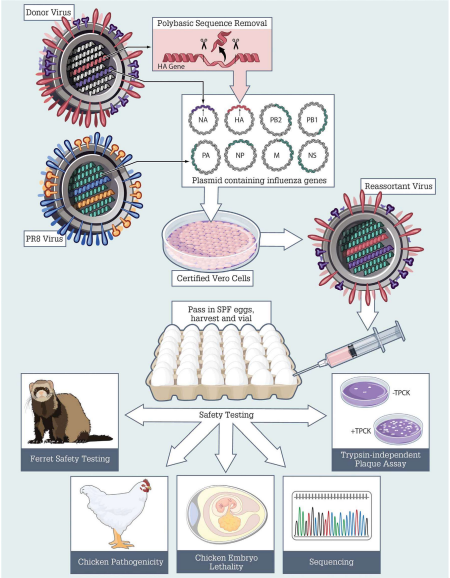
Mouse serum										
	Alum (30 mg)					AddaVax				
Antigens	NIBRG-268	NHRI-RG3	NHRI-RG4	NHRI-RG5	NHRI-RG6	NIBRG-268	NHRI-RG3	NHRI-RG4	NHRI-RG5	NHRI-RG6
NIBRG-268	508.0	160.0	89.8	285.1	144.9	579.7	237.8	237.8	320.0	359.2
NHRI-RG3	113.1	71.3	25.2	127.0	80.0	118.9	118.9	88.3	176.7	118.9
NHRI-RG4	142.5	63.5	80.0	201.6	80.0	160.0	107.7	262.5	289.8	131.3
NHRI-RG5	142.5	80.0	25.2	226.3	44.2	201.4	118.9	118.9	262.5	118.9
NHRI-RG6	80.0	71.3	20.0	113.1	88.3	118.9	131.3	80.0	195.0	160.0



Virology 511 (2017) 135–141.

Chicken embryo lethality test

Viruses	Pathogenicity	$CELD_{50}$
Wild type H7N9 (A/Taiwan/1/2017)	HPAI	<1.7E+02 $TCID_{50}$
NHRI-RG2 (A/Anhui/1/2013)	HPAI	>2.50E+07 $TCID_{50}$
NHRI-RG3 (A/Guangdong/17SF003/2016)	HPAI	>4.45E+06 $TCID_{50}$
NHRI-RG4 (A/Hong Kong/125/2017)	LPAI	>1.06E+07 $TCID_{50}$
NHRI-RG5 (A/Guangdong/SP440/2017)	HPAI	>4.45E+07 $TCID_{50}$
NHRI-RG6 (A/Taiwan/1/2017)	HPAI	>4.45E+06 $TCID_{50}$

Plaque-forming ability of H7N9 reassortant viruses in MDCK cells with or without trypsin

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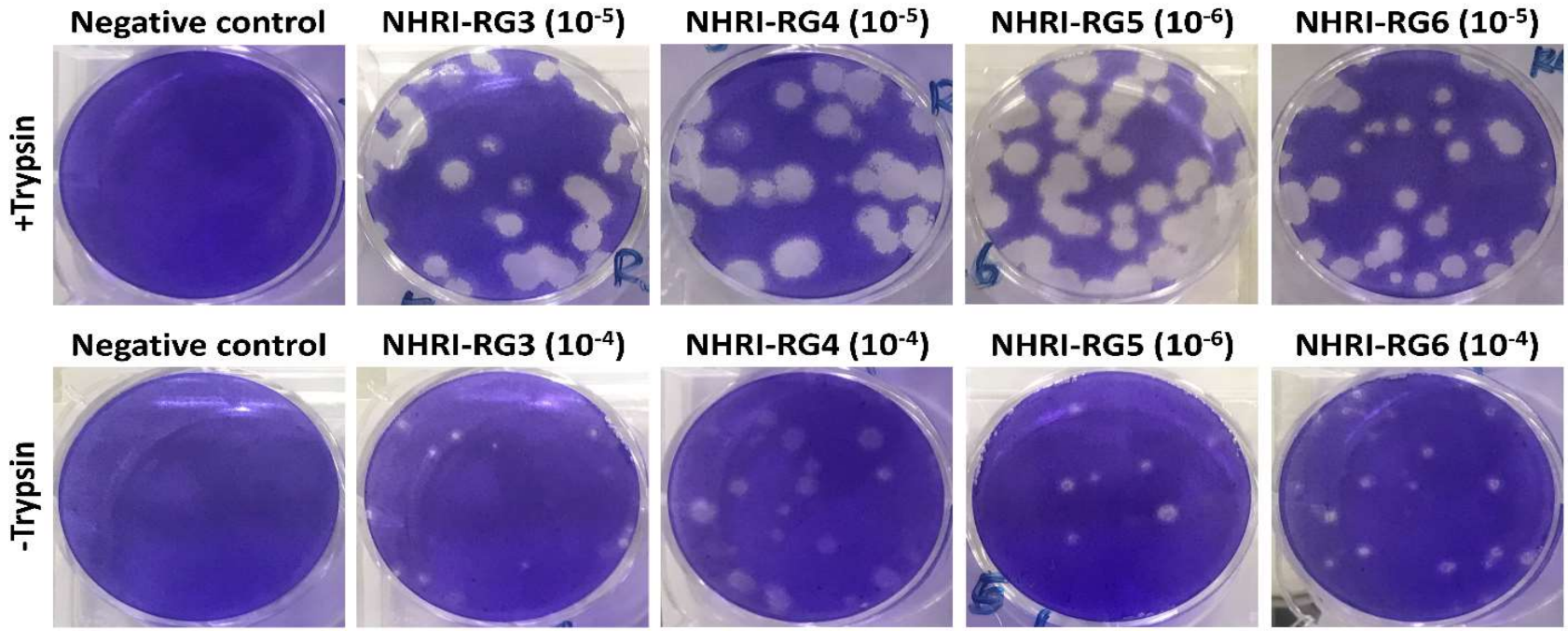

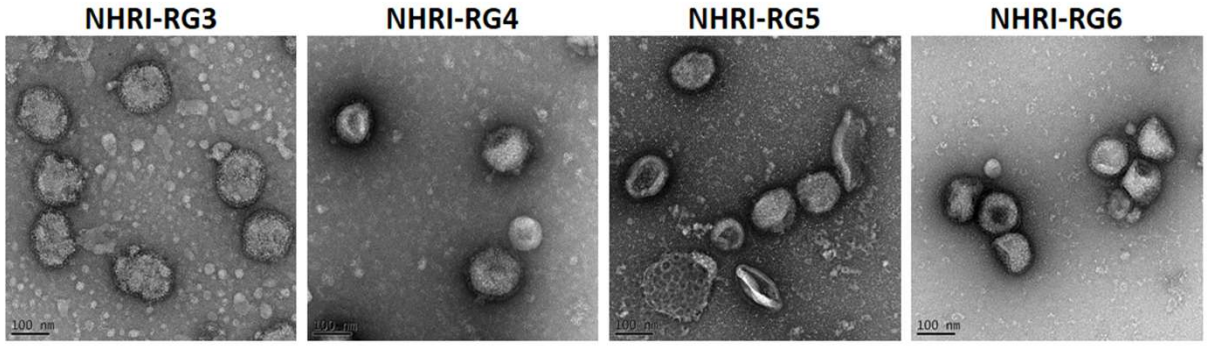

Medium cost estimation in the USP

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	aMDCK (H7N9)	aMDCK (H7N9)	sMDCK 3rd DSP	egg_based
Medium	Opti-Pro	BalanCD MDCK	BalanCD simple	
HA titer	438.1	512.0	1160	
Medium required ratio (medium usage/harvest volume)	3.1	3.1	2.0	
one dose required HA (ug)	15	15	15	
downsteam recovery rate	0.3	0.3	0.7	
US $ /dose	3.5x2	1.51x2	0.22x2	0.5~0.8

US Patent filed (#62248954)
PCT Patent filed (WO2017072744 (A1))
ROC Patent filed (TW201726911 (A))

16X reduction

1L= ~200 doses
1000L= ~200,000 doses
Based on 30 µg/dose

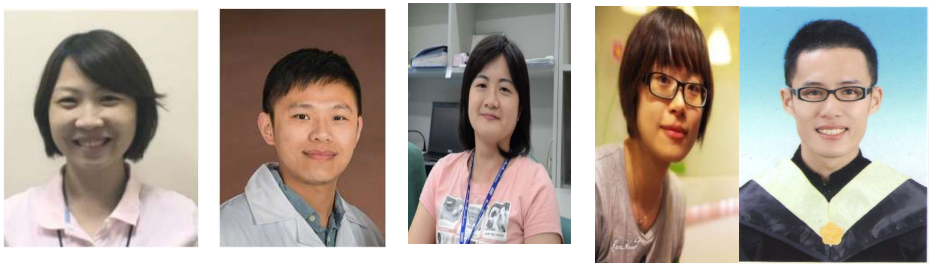
Segirus

Suspension MDCK-33016 cells
Serum-free medium
150 millions/year
US$ 1 billions
Holly Springs, USA

Acknowledgements

- VC & NIIDV directors
  - Dr. Pele Chong
  - Dr. I-Jen Su
  - Dr. I-Chun Chen
  - Dr. Ching-len Liao
- NIIDV collaborators
  - Dr. Min-Shi Lee
  - Dr. Min-Shi Huang
  - Dr. Jen-Ren Wang
  - Dr. Jerry Sung
- Bioproduction
- Worldwide collaborators
  - US CDC/BARDA
  - JP NIID
  - TW CDC
  - UK NIBSC
  - AU VIDRL
  - TW MOST/MOHW
  - US Irvine Scientific
  - TW Medigen
  - Tantii
  - Merck

Dr. Cheng Postdoc | Dr. Tseng Postdoc | May RA | PinWen RA | SinYi RA | SinYi RA | Chia-Chun RA | Chia-Chun PhD | ChunHsiang MSc



Tantti 2.0 春酒