

PENGGUNAAN TEKNOLOGI REVERSE GENETICS

dalam
PEMBUATAN VAKSIN AI

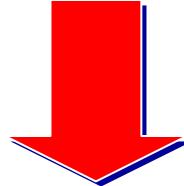
drh. Kamaluddin Zarkasie, PhD.



SEJARAH VAKSINASI

200 tahun semenjak Edward Jenner

Vaksin dpt mengatasi penyakit : smallpox, diphteria, tetanus, yellow fever, pertussis, Hib, poliomyelitis, measles, mumps,& rubella.



vaksinasi berdampak besar dalam menurunkan jumlah kematian akibat penyakit

VAKSIN

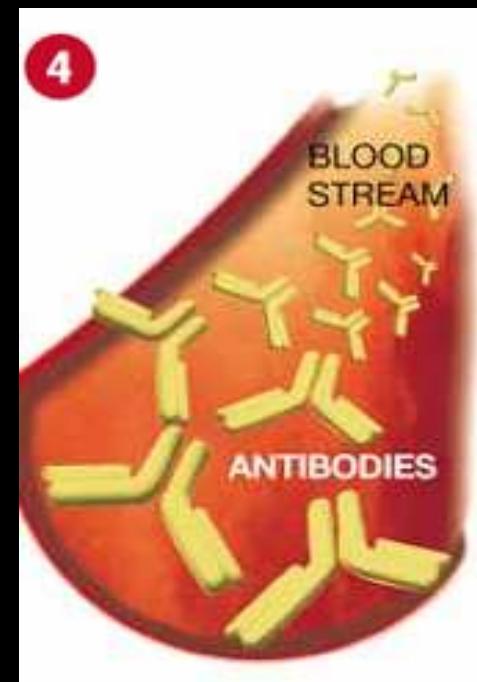
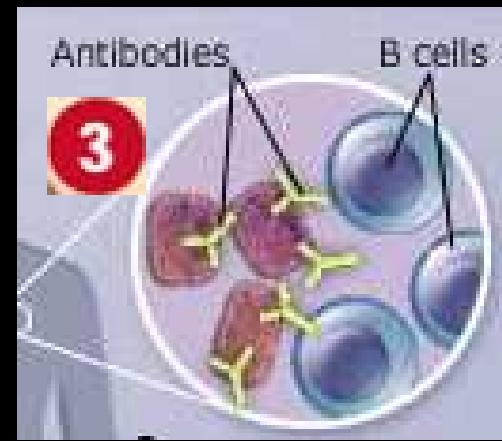
- Asal Kata : 'Vaccinia'
- Definisi :
 1. Sediaan biologik yang digunakan untuk menimbulkan kekebalan terhadap suatu penyakit hewan (Penjelasan PP Nomor 78 tahun 1992 tentang Obat Hewan)
 2. Bahan antigenik yang digunakan untuk menghasilkan kekebalan aktif terhadap suatu penyakit sehingga dapat mencegah atau mengurangi pengaruh infeksi oleh organisme alami atau 'liar'.



Vaksinasi

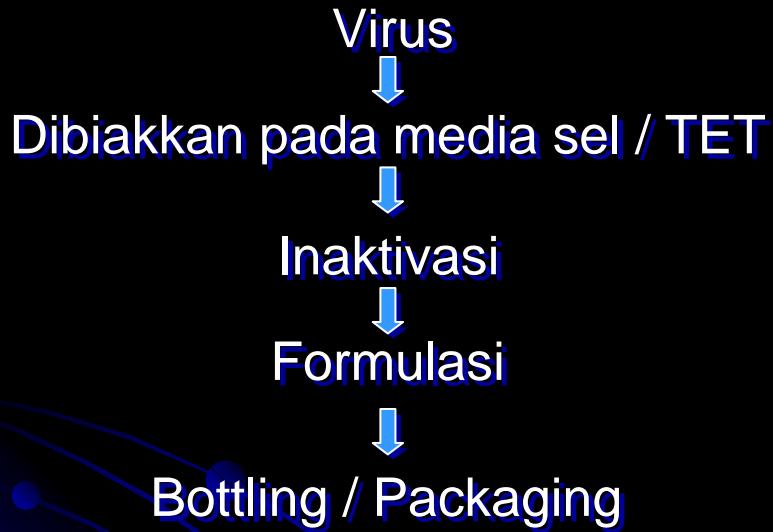
Pemberian vaksin kedalam tubuh manusia atau hewan untuk memberikan kekebalan terhadap suatu penyakit.



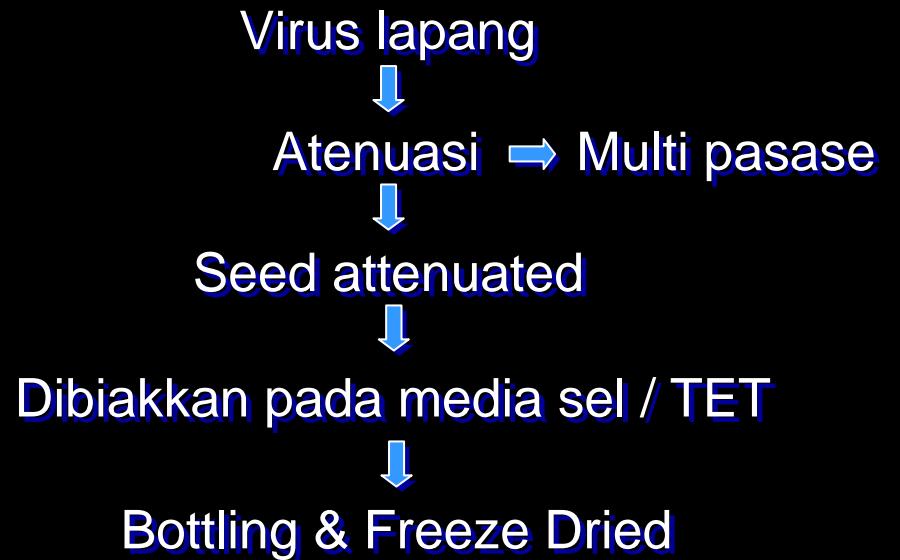


Teknik Produksi Vaksin

KONVENSIONAL VAKSIN INAKTIF



KONVENSIONAL LIVE VAKSIN





Comparative Properties of Active Vaccines

Characteristics	Advantages	Challenges
LIVE VACCINES		
<ul style="list-style-type: none">■ Able to replicate in the host■ Attenuated in pathogenicity■ Elicit antibodies and cell-mediated immunity	<ul style="list-style-type: none">■ May elicit broader immune responses■ May require fewer doses■ Generally longer lasting protection	<ul style="list-style-type: none">■ Uncertain window for attenuation■ Uncertain safety before large-scale use■ Stability■ Analysis
INACTIVATED VACCINES		
<ul style="list-style-type: none">■ Unable to replicate in the host■ Elicit mostly antibodies	<ul style="list-style-type: none">■ Cannot multiply or revert to pathogenicity■ Generally less reactogenic■ Nontransmissible to another person■ Usually more feasible technically	<ul style="list-style-type: none">■ May require adjuvant■ May require delivery system■ Immunogenic potency■ Variable efficacy
GENETIC VACCINES (DNA BASED)		
<ul style="list-style-type: none">■ Stimulate synthesis of antigens only in cells■ Elicit mostly cell-mediated immunity	<ul style="list-style-type: none">■ Standardized method of production and analysis■ Sustained immunologic stimulation	<ul style="list-style-type: none">■ Establishing proof-of-principle■ Immunogenic potency

Live vaccines and their approximate times of availability

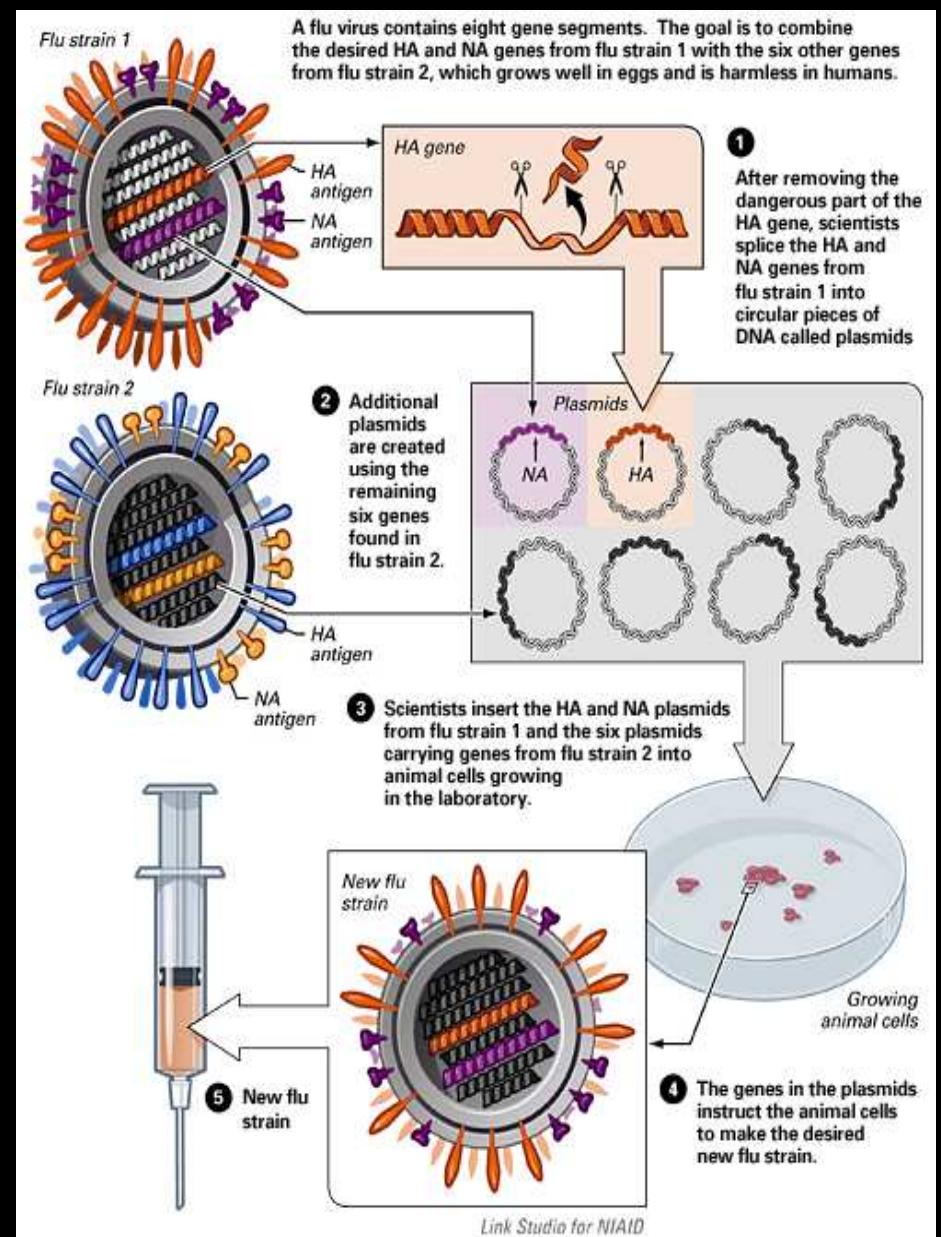
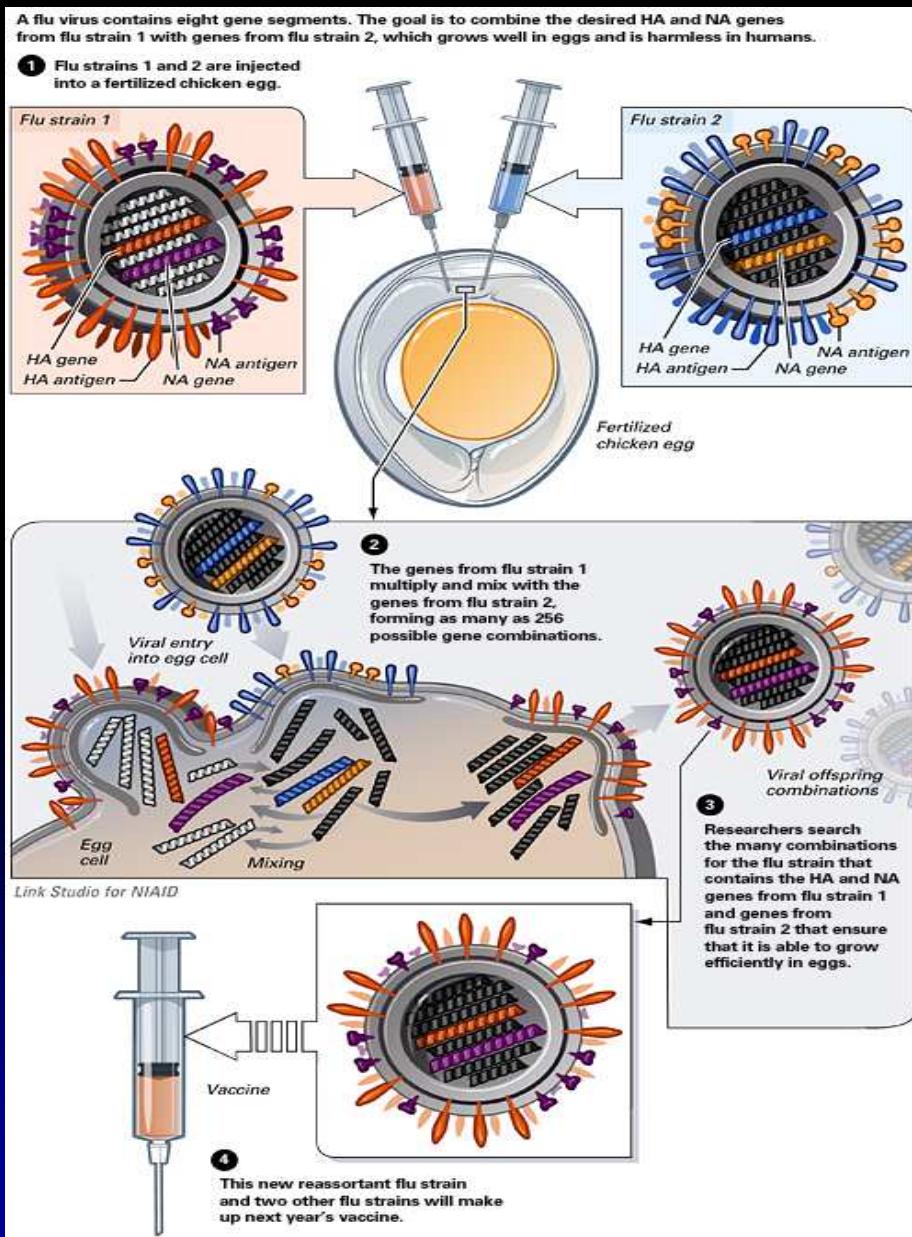
Development strategy	Date	Vaccine or target
Use of related animal virus	1798	Smallpox
Chemical attenuation	1885	Rabies
	1881	Anthrax
Passage <i>in vitro</i>	1927	BCG
	1935	Yellow fever
Cell culture passage	1962	Oral polio vaccine
	1963	Measles
	1971	Adenoviruses
	1995	Varicella
	2005	Rotavirus 89-12
Cell culture passage with cold adaptation	1969	Rubella
	2003	Live influenza
Auxotrophy	1989	Ty21a typhoid
Use of reassortants	1970s	Inactivated influenza seed
	2003	Live influenza

Nonliving vaccines and their approximate times of availability

Vaccine strategy	Date	Vaccine or target
Inactivated whole organisms	1896	Typhoid
	1896	Cholera
	1897	Plague
	1926	Whole-cell pertussis
	1938	Influenza
	1955	Injectable Polio Vaccine
	1995	Hepatitis A
Use of extracts and subunits	1944	Japanese encephalitis
	1970s	Influenza
	1960	Anthrax
	1976	Cell-culture rabies
Use of toxoids	1923	Diphtheria
	1927	Tetanus
	2008 (?)	New anthrax

Newer strategies for vaccine development starting from microbial DNA, cDNA or RNA	
Strategy	Examples of pathogens targeted
Recombinant protein production	Hepatitis B S_{Ag}, pertussis toxin, Lyme outer surface protein A, CMV gB protein
Live recombinants carrying genes from related agents	Dengue genes in yellow fever 17D, parainfluenza 1 + 2 genes in parainfluenza 3, <i>M. tuberculosis</i> genes in BCG
Recombinant vectors recombining genes from pathogens	HIV, CMV
Alpha virus replicons	HIV, Hemorrhagic Fevers
Replication-defective particles	HPV, SARS
'Naked' DNA plasmids	HIV and many others
Prime boost using DNA and/or vectors	HIV, malaria, tuberculosis
Reverse vaccinology	Meningococcus B
Microarrays for expression of virulence genes	Mainly bacteria
Synthetic peptides	Cancer, CTL vaccines
Synthetic capsular polysaccharides	Hib
Reverse genetics	Influenza, parainfluenza, RSV
Hib, <i>H. influenza</i> type b; IPV, inactivated polio vaccine; T, tetanus; d, adult diphtheria dose; CMV, cytomegalovirus; HPV, human papillomavirus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; HIV, human immunodeficiency virus; CTL, cytotoxic T lymphocyte.	

Ilustrasi Reassortment Vs Reverse Genetics



Current Influenza Vaccines

- WHO global surveillance → strain selection
- Trivalent inactivated vaccine – A/H3N2, A/H1N1 and B viruses
- Reference strains prepared in eggs in WHO Collaborating Center laboratories using genetic reassortment
 - H and N genes from wild-type virus
 - 6 genes from high-growth lab strains
- Given free-of-charge to all vaccine companies
- Production cycle – 6 to 8 months
- Vaccine cannot be stockpiled beforehand

Reverse Genetics of Influenza Viruses

- Developed by Palese, et al. (1989) and modified by several other groups
- Wild-type RNA for H and N genetic segments cloned into plasmids
- Transferred to cell culture with helper and backbone (the other 6 genes) plasmids
- Infectious supernate inoculated into embryonated eggs to prepare reference strains

R&D for Avian / Pandemic Influenza Vaccines by IFPMA Influenza Vaccine Supply International Task Force (IVS ITF) members
 (Updated 24 January 2006)

Company	Strain	Type	Culture	Adjuvant	Doses (µg)	Trials	Timing	Comments
Baxter	H5N1 wild type (A/Vietnam/1203/2004)	Inactivated whole virion	Cell (Vero)	Alum	3.7 / 7.5 / 15 / 30 / 45	Ph I & II	Starts Q2 2006	
Berna Biotech + Leicester University	H9N2	Inactivated whole virion	Egg	Alum	1.7 / 5 / 15 / 45	Ph II	Starts early 2006	Intramuscular+subcutaneous 2 shots, 3 weeks apart
Berna Biotech + Leicester University	H9N2	Virosomal	Egg	none	1.7 / 5 / 15 / 45	Ph II	Starts early 2006	Intramuscular+subcutaneous 2 shots, 3 weeks apart
Biken	H5N1 (NIBRG-14)	Inactivated whole virion	Egg	Alum	1.7 / 5 / 15	Ph I	Q1-2 2006	Intramuscular+subcutaneous Ph II & III Q3-4 2006
Chiron	H9N2 (G9/PR8)	Inactivated surface antigen	Egg	MF59	3.75 / 7.5 / 15 / 30			Unpublished results
Chiron	H5N1	Inactivated surface antigen	Egg	MF59	7.5 / 15	Ph II	2006	
Chiron	H5N3 (A/duck/Singapore/1997, NIB 40)	Inactivated surface antigen	Egg	MF59	7.5 / 15 / 30	Yes	Done	Lancet 2001 357: 1937-1943 Vaccine 2003 21: 1687-1693 JID 2005 191: 1210-1215
Chiron	H5N1	Inactivated surface antigen	Egg	MF59	7.5 / 15	Proof of concept	Starts early 2006	
Chiron	H5N1	Inactivated surface antigen	Egg	Alum	7.5 / 15 / 30 / 60	Proof of concept	Starts early 2006	
CSL	H5N1 (NIBRG-4)	Split virus inactivated	Egg	AlPO ₄	7.5 / 15	Yes	Results Feb 2006	2 nd trial to finish end 2006, depending on results of 1st
Denka Seiken	H5N1 (NIBRG-14)	Inactivated whole virion	Egg	Alum	1.7 / 5 / 15	Ph I	Q1-2 2006	Intramuscular+subcutaneous Ph II & III Q3-4 2006

© 2006 IFPMA - Note: where an adjuvant is employed, standard practice is to evaluate it against the same vaccine without an adjuvant. Where alum is indicated as adjuvant, this refers to aluminium hydroxide Al(OH)₃ or a mixture of aluminium hydroxide and aluminium phosphate AlPO₄. Where aluminium phosphate is used on its own, it is indicated as AlPO₄. The dose, in µg, refers to the volume of haemagglutinin (HA).

R&D for Avian / Pandemic Influenza Vaccines by IFPMA Influenza Vaccine Supply International Task Force (IVS ITF) members
 (Updated 24 January 2006)

Company	Strain	Type	Culture	Adjuvant	Doses (µg)	Trials	Timing	Comments
GSK Biologicals	H2N2	Whole virion	Egg	Alum	1.9 / 3.8 / 7.5 / 15			Mock-up file submitted 21 Dec 2005
GSK Biologicals	H9N2	Whole virion	Egg	Alum	1.9 / 3.8 / 7.5 / 15			Mock-up file submitted 21 Dec 2005
GSK Biologicals	H5N1	Whole virion	Egg	Alum	1.9 / 3.75 / 7.5 / 15			Ex-ID Biomedical
Kaketsukan	H5N1 (NIBRG-14)	Inactivated whole virion	Egg	Alum	1.7 / 5 / 15	Ph I	Q1-2 2006	Subcutaneous Ph II & III Q3-4 2006
Kitasato Institute	H5N1 (NIBRG-14)	Inactivated whole virion	Egg	Alum	1.7 / 5 / 15	Ph I	Q1-2 2006	Intramuscular+subcutaneous Ph II & III Q3-4 2006
MedImmune	H5N1 A/Vietnam/1203/2004	Live attenuated	Egg	none	Intranasal	yes	Starts 2006	
MedImmune	H5N1 A/HongKong/492/1997	Live attenuated	Egg	none	Intranasal	yes	Starts 2006	
MedImmune	H9N2	Live attenuated	Egg	none	Intranasal	Ph I	Ended 2005	
MedImmune	H5N1	Live attenuated	Cell	none	Intranasal	Proof of concept	2006	
Nobilon	H5N1 (NIBRG-14)	Whole virion inactivated	Cell	Alum		yes	Starts 2 nd half 2006	
sanofi pasteur	H5N1 (France)	Split virus inactivated	Egg	Alum	7.5 / 15 / 30	Ph II	2006	Ph I completed 2005
sanofi pasteur	H5N1 (US - NIAID)	Split virus inactivated	Egg	none	7.5 / 15 / 45 / 90	Ph I elderly	ongoing	Ph I adult completed 2005
sanofi pasteur	H5N1 (US - NIAID)	Split virus inactivated	Egg	Alum	7.5 / 15 / 45	Ph I adult	2006	
sanofi pasteur	H7N7 (US - NIAID)	Split virus inactivated	Egg	Alum		Ph I adult	2006	

© 2006 IFPMA - Note: where an adjuvant is employed, standard practice is to evaluate it against the same vaccine without an adjuvant. Where alum is indicated as adjuvant, this refers to aluminium hydroxide Al(OH)₃ or a mixture of aluminium hydroxide and aluminium phosphate AlPO₄. Where aluminium phosphate is used on its own, it is indicated as AlPO₄. The dose, in µg, refers to the volume of haemagglutinin (HA).

R&D for Avian / Pandemic Influenza Vaccines by IFPMA Influenza Vaccine Supply International Task Force (IVS ITF) members
 (Updated 24 January 2006)

Company	Strain	Type	Culture	Adjuvant	Doses (µg)	Trials	Timing	Comments
sanofi pasteur	H7N1 (EU - Flupan)	Split virus inactivated	Cell	Alum		Ph I adult	2006	Trial under Flupan sponsorship
Solvay	H5N1 (NIBRG-14)	Inactivated subunit	Egg	Alum		yes	Starts Q3 2006	
Solvay	H5N1 (NIBRG-14)	Inactivated subunit	Cell	Alum		yes	Starts Q3 2006	

About the Pandemic R&D table: The table lists all the prototype avian / pandemic influenza vaccines under development by IFPMA Influenza Vaccine Supply International Task Force member companies. As such, it covers the vast majority of R&D projects being undertaken in this field. The number of projects and the commitment of these companies to undertake the necessary clinical trials underline the vaccine industry's commitment to help minimise the global health impact of avian influenza and any related pandemic influenza.

About IFPMA (www.ifpma.org): The International Federation of Pharmaceutical Manufacturers & Associations is a non-profit, non-governmental Organization (NGO) representing national industry associations and companies from both developed and developing countries. Member companies of the IFPMA are research-based pharmaceutical, biotech and vaccine manufacturers.

About IFPMA Influenza Vaccine Supply International Task Force: The IFPMA Influenza Vaccine Supply Interventional Task Force was established in February 2002, bringing together research based influenza vaccine manufacturers from Australia, Europe, Japan and North America. The Task Force works within the constraints of anti-trust law to address advocacy, communication, policy, regulatory, scientific and technical issues related to interpandemic and pandemic influenza vaccines. Members manufacturers are: Baxter Vaccines, Berna Biotech, Biken, Chiron Vaccines, CSL Ltd, Crucell, Denka Seiken, GlaxoSmithKline Biologicals (including ID Biomedical), Kaketsuken, Kitasato Institute, MedImmune, Nobilon, sanofi pasteur, Sanofi Pasteur MSD and Solvay Pharmaceuticals.

A backgrounder entitled Preparing for pandemic influenza vaccination can be found at (http://www.ifpma.org/pdf/pandemic_backgrounder_23Jan06.pdf)

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© 2006 IFPMA - Note: where an adjuvant is employed, standard practice is to evaluate it against the same vaccine without an adjuvant. Where alum is indicated as adjuvant, this refers to aluminium hydroxide Al(OH)₃ or a mixture of aluminium hydroxide and aluminium phosphate AlPO₄. Where aluminium phosphate is used on its own, it is indicated as AlPO₄. The dose, in µg, refers to the volume of haemagglutinin (HA).

Aplikasi Teknologi Reverse Genetics

1) Mekanisme replikasi virus

Uncoating

Replikasi RNA

Transkripsi

Translasi

Packaging

2) Vektor virus Influenza

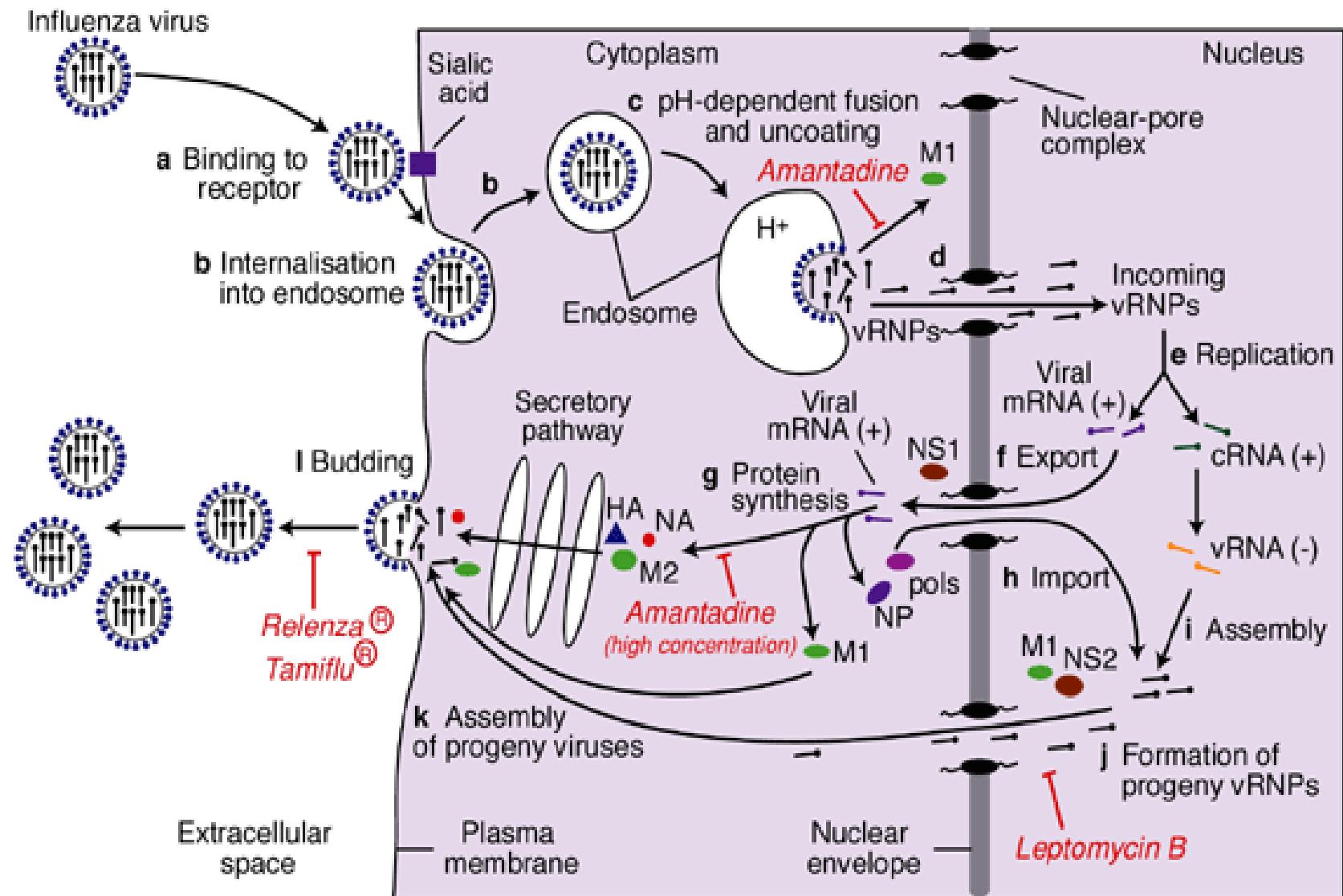
HIV gp41, nef

M. tuberculosis Ag85A, ESAT6

PR8

3) Vaksin Rekombinan

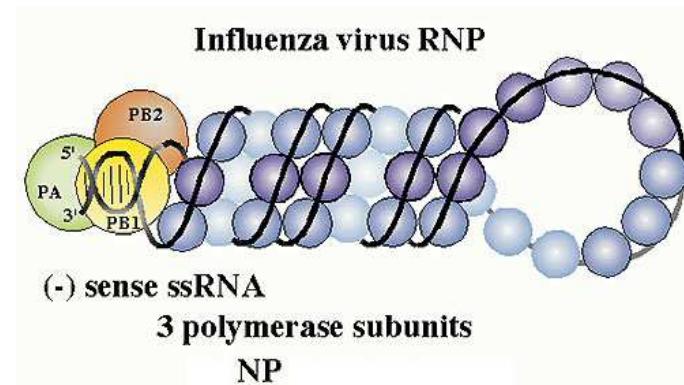
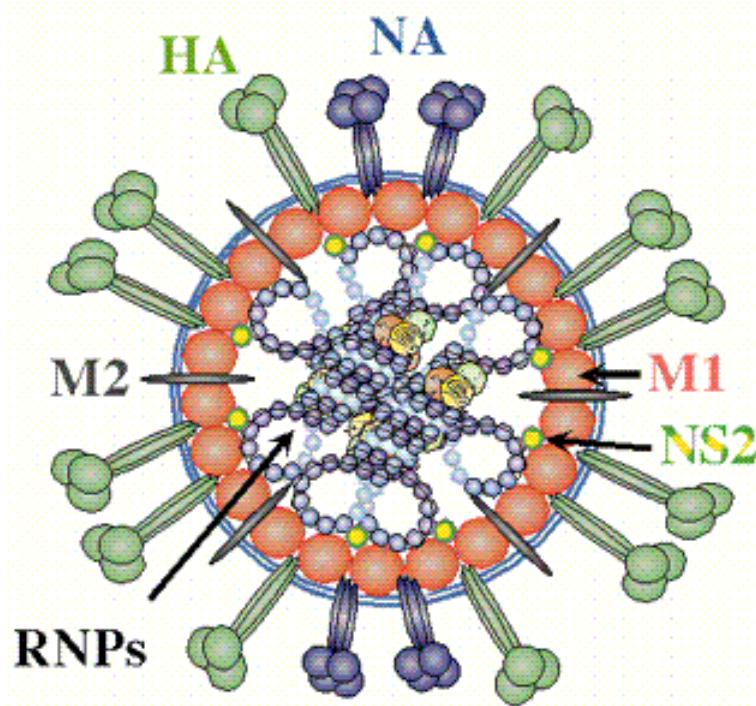
Mekanisme Replikasi Virus pada Sel Inang



Replication cycle of an influenza virus

Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

Struktur Virus AI



The genome segments are packaged into the core. The **RNP** (RNA + nucleoprotein, N) is in a helical form with the **3 polymerase polypeptides** associated with each segment.

The outer surface of the particle consists of a lipid **envelope** from which project prominent **glycoprotein spikes** of two types:

- **haemagglutinin (HA)**, a 135Å trimer
- **neuraminidase (NA)**, a 60Å tetramer

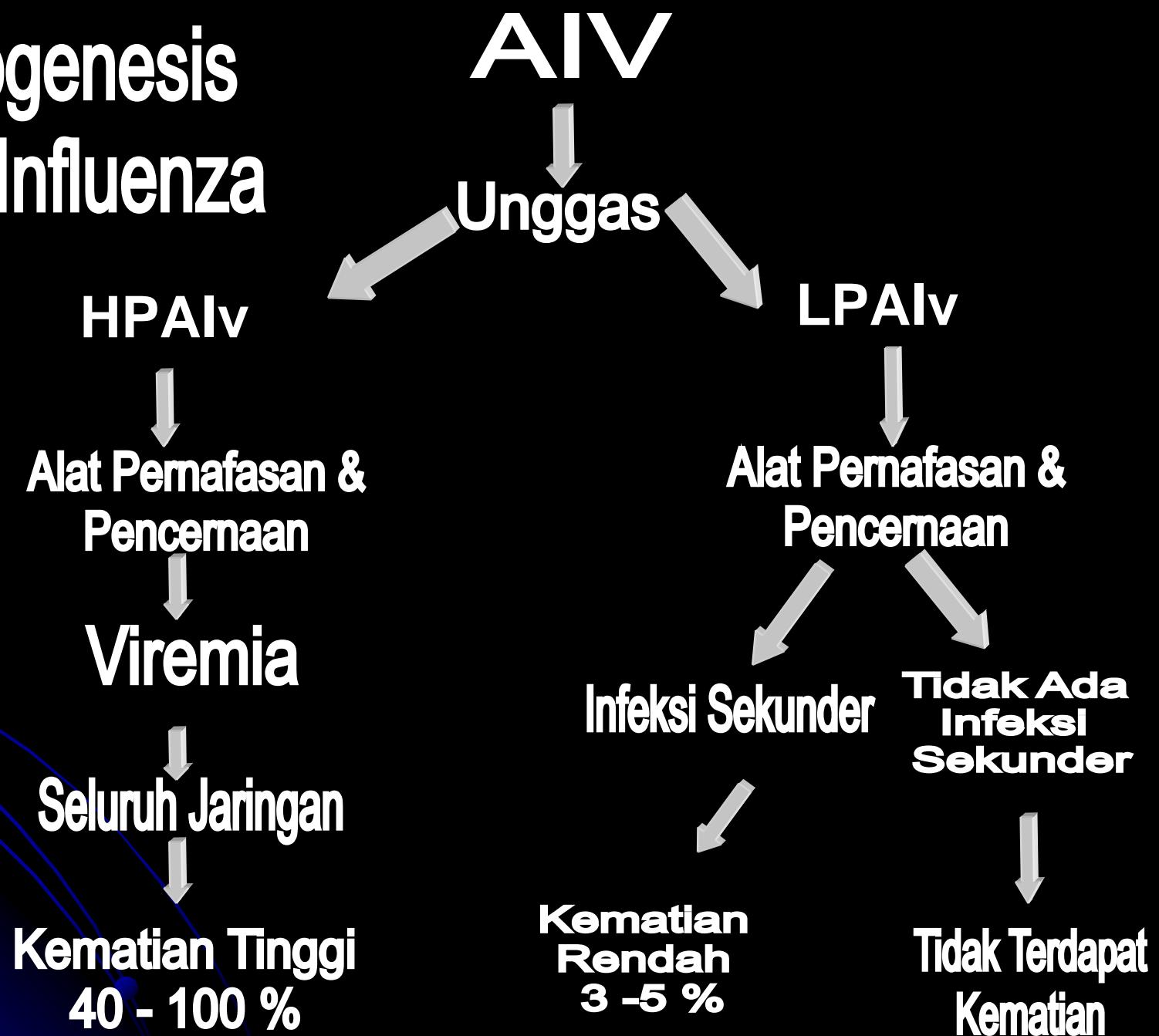
The inner side of the envelope is lined by the **matrix protein**.

The particles are relatively labile (half-life a few hours @ R.T.), not resistant to drying, etc.

Struktur Protein Virus AI

Segment:	Size(nt)	Polypeptide(s)	Function
1	2341	PB2	Transcriptase: cap binding
2	2341	PB1	Transcriptase: elongation
3	2233	PA	Transcriptase: protease activity (?)
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex; nuclear/cytoplasmic transport of vRNA
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8	890	NS1	Non-structural: nucleus; effects on cellular RNA transport, splicing, translation. Anti-interferon protein.
		NS2	Non-structural: nucleus+cytoplasm, function unknown

Pathogenesis Avian Influenza



Strategi Pengendalian AI

- Identifikasi Subtipe Virus
- Peningkatan Biosekuriti
- Perlindungan daerah bebas AI dari penularan
- Depopulasi pada daerah tertular
- Pembatasan pergerakan unggas dan produknya
- Pengendalian transportasi limbah peternakan
- Vaksinasi strategis
- Epidemiological surveillance

Vaksin AI Yang Telah Mendapat izin di Indonesia

No	Nama Vaksin	Produsen	Importir	Jenis Vaksin	Seed
1	Afluvet	Pusvetma	- (lokal)	Homolog inaktif	High Pathogenic AI H5N1
2	Vaksiflu AI	PT. Vaksindo	- (lokal)	Homolog inaktif	High Pathogenic AI H5N1
3	Medivac AI	PT. Medion	- (lokal)	Homolog inaktif	High Pathogenic AI H5N1
4	Inactivated AI VAC Oil Emulsion	Qilu AH Products Fact, China	PT. Bio Farma	Homolog inaktif	High Pathogenic AI H5N1
5	Avian Influenza Killed Virus Vaccine	Laboratorio Avi-Mex, SA de CV, Mexico	PT. Avindo PBM	Heterolog inaktif	Low Pathogenic AI H5N2
6	Volvac AI	Boehringer Ingelheim Vetmedica, SA De CV, Mexico	PT. Boehringer Ingelheim Indonesia	Heterolog inaktif	Low Pathogenic AI H5N2
7	Optimune AI KV	Biomune de Mexico SA de CV, Mexico	PT. Agrinusa Unggul Jaya	Heterolog inaktif	Low Pathogenic AI H5N2
8	Nobilis Influenza H5	Intervet int'nal, Belanda	PT. Intervet Indonesia	Heterolog inaktif	Low Pathogenic AI H5N2
9	Gallimune Flu H5N9	Merial, Italia	PT. Romindo Primavetcom	Heterolog inaktif	Low Pathogenic AI H5N9
10	Bird Close5.1	IPB-SGT	-(lokal)	Homolog inaktif	Non Pathogenic AI H5N1

Sumber : Ditjenak Deptan

Vaksinasi pada Unggas

- Mampu mengurangi **MORBIDITAS** dan **MORTALITAS** pada unggas
- Mengurangi **KONTAMINASI** lingkungan
- *Tapi BILA CAKUPAN VAKSINASI TIDAK BAIK, akan MENIMBULKAN dan MENINGKATKAN JUMLAH UNGGAS RESERVOIR (subclinical & virus shedding)*



Teknologi Produksi Vaksin

1. Vaksin Konvensional

- Vaksin homolog inaktif
- Vaksin heterolog inaktif

2. Vaksin Rekombinan

- Vaksin rekombinan aktif dengan vektor virus lain
- Vaksin rekombinan inaktif ‘reverse genetic’

Teknologi Reverse Genetics

- Menghilangkan bagian patogen dari virus H5N1 sehingga virus yang dihasilkan bersifat **NON-PATOGENIK**
- Virus yang dihasilkan **AMAN** untuk produksi skala besar pada fasilitas BSL 2 (Ketentuan WHO "Production of inactivated influenza vaccines from reassortants derived from AI viruses", 2003)
 - Antigen yang dihasilkan memiliki **homologi** asam amino ~ 100% dengan isolat lapang
 - Menghasilkan **titer antibodi yang tinggi dan konsisten**
 - hemat waktu

Sumber : WHO, Dept. of Communicable Disease Surveillance and Response

REGULASI

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Part 2 Chapter 2.1.14.C :

1. Vaksin AI aktif konvensional tidak direkomendasikan penggunaannya
2. Vaksin AI inaktif konvensional hanya boleh diproduksi dengan menggunakan *seed virus* *low pathogenic*.



GLOBAL
HEALTH
SECURITY

EPIDEMIC
ALERT &
RESPONSE

**Production of pilot lots of
inactivated influenza vaccines
from reassortants derived from
avian influenza viruses**

Interim biosafety risk assessment



DEPARTMENT OF COMMUNICABLE DISEASE
SURVEILLANCE AND RESPONSE

© World Health Organization 2003

http://www.who.int/csr/resources/publication/influenza/influenzaRMD2003_5.pdf

WHO Technical Report Series No. 927, 2005

ANNEX 3

RECOMMENDATIONS FOR PRODUCTION AND CONTROL OF INFLUENZA VACCINE (INACTIVATED)

Introduction.....

General considerations.....

Part A

Manufacturing recommendations.....

A1 Definitions.....

A2 General manufacturing recommendations.....

A3 Production control.....

A4 Filling and containers.....

A5 Control tests on final lot.....

A6 Records.....

A7 Retained samples.....

A8 Labelling.....

A9 Distribution and transport.....

A10 Stability testing, storage and expiry date.....

Part B

Recommendations for National Control Authorities.....

B1 General.....

B2 Release and certification.....

B3 Clinical evaluation of influenza vaccines.....

Authors.....

Acknowledgements.....

It is now common practice to use reassortant strains give high yields of the appropriate surface antigens. Reassortant strains for vaccine production have the surface glycoproteins (haemagglutinin and neuraminidase) of the recommended reference virus and internal proteins of a high growth donor virus. These recommendations shall also apply to the subsequent production and quality control of reassortant vaccine viruses produced by reverse genetics.

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RECOMMENDATIONS FOR PRODUCTION AND CONTROL OF
INFLUENZA VACCINE (INACTIVATED) page 105

Where reverse genetics is used to generate the reassortant vaccine virus, the influenza haemagglutinin and neuraminidase genes may be derived from a variety of sources.

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RECOMMENDATIONS FOR PRODUCTION AND CONTROL OF
INFLUENZA VACCINE (INACTIVATED) page 111

The H5N1xPR8 reassortants created in this project will not contain the gene constellation considered necessary for pathogenicity in chickens, mice and ferrets.

Reassortants derived from PR8 have been used routinely for production of inactivated influenza vaccines for the past 30 years. This work involves production of many thousands of litres of infected egg allantoic fluids, which will create substantial aerosols of reassortant virus within manufacturing plants. Most of the reassortants were made from wild-type human strains that had not yet been in widespread circulation. Thus, although the manufacturing staff would have some susceptibility to infection with the wild-type virus, there have been no anecdotal or documented cases of work-related human illness resulting from exposure to the reassortants. This is further testimony to the attenuation of PR8 reassortants. Nevertheless, unlike the situation with normal vaccine production, manufacturing staff for pilot lots of a potential pandemic vaccine would have no previous immunological experience of an avian virus, so staff would be expected to be susceptible, although the risk is expected to be low.

Genetic stability of reassortant viruses is an important issue as the wild-type non-pathogenic H5 and H7 avian viruses are the source of highly pathogenic viruses. Studies of a non-pathogenic H5N3 reassortant between A/Goose/Hong Kong/437/99 and PR8 have shown no evidence of reversion to virulence (chickens, mice and ferrets) after 10 passages in eggs (R Webster, unpublished data).

Production of pilot lots of inactivated influenza vaccines from reassortants derived from avian influenza viruses

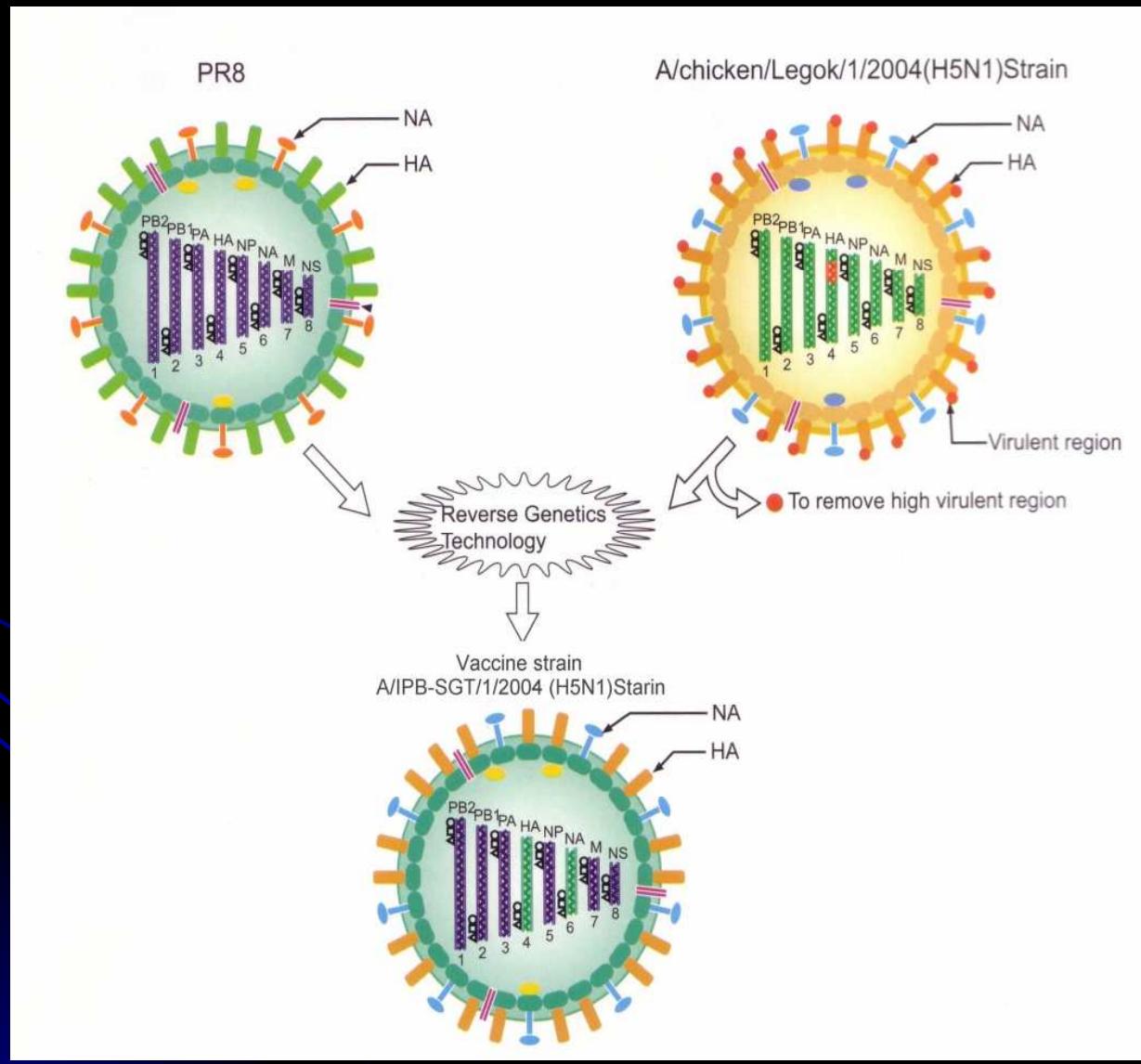
WHO Interim biosafety risk assessment, Halaman 5.

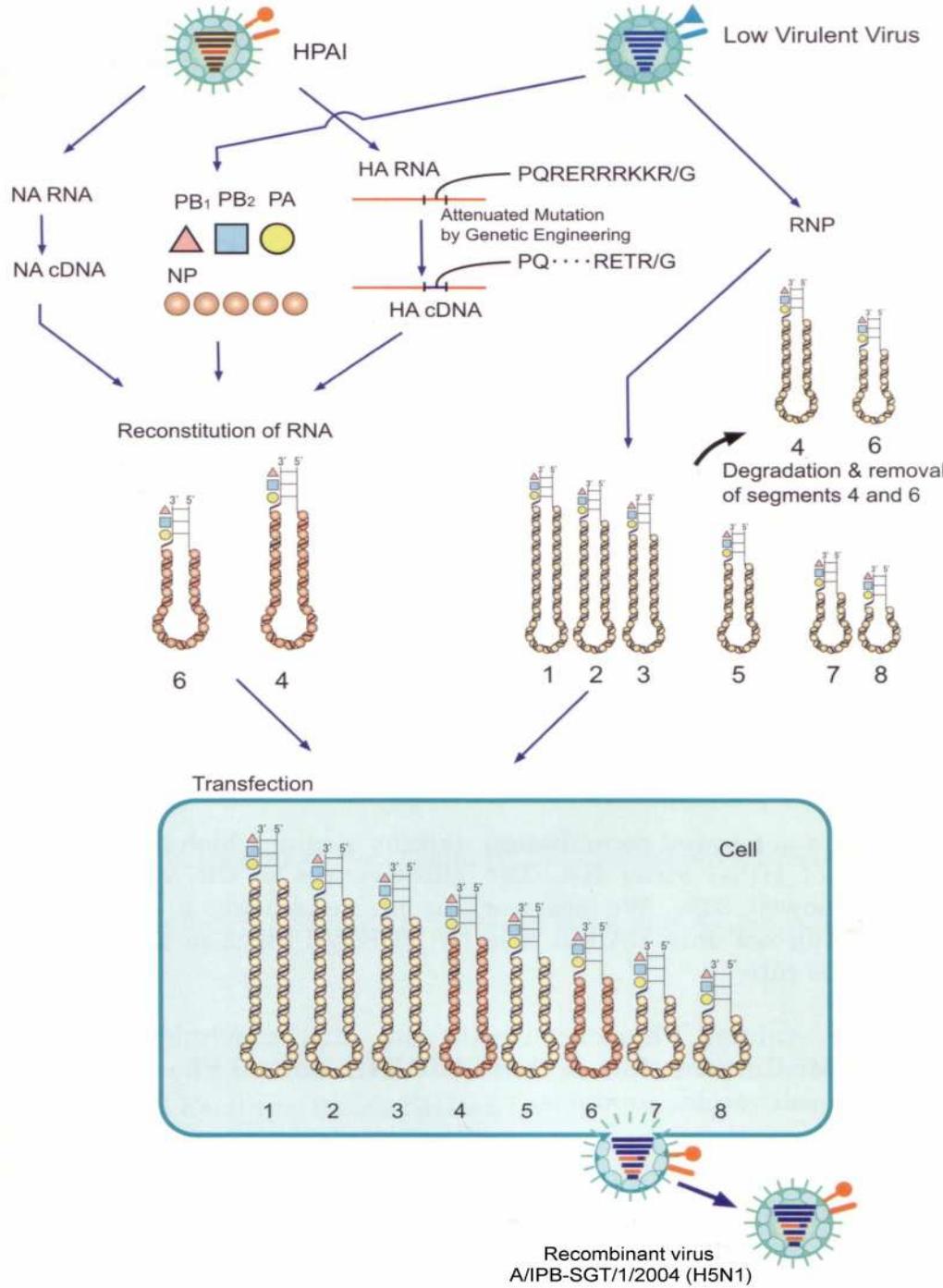
The important features of reverse genetics for pandemic influenza vaccine development are as follows:

- Ability to genetically modify a highly pathogenic avian virus so that the molecular basis for pathogenicity is removed. This dramatically reduces the danger associated with the virus.
- Ability to produce reassortants between a modified safe avian virus and a human vaccine virus such as A/PR/8/34. A PR8 reassortant will grow well in mammalian cells and in eggs and it is likely to be attenuated for man, thus improving the safety profile of a pandemic vaccine virus.

Sumber: <http://www.eurosurveillance.org/ew/2004/040617.asp>

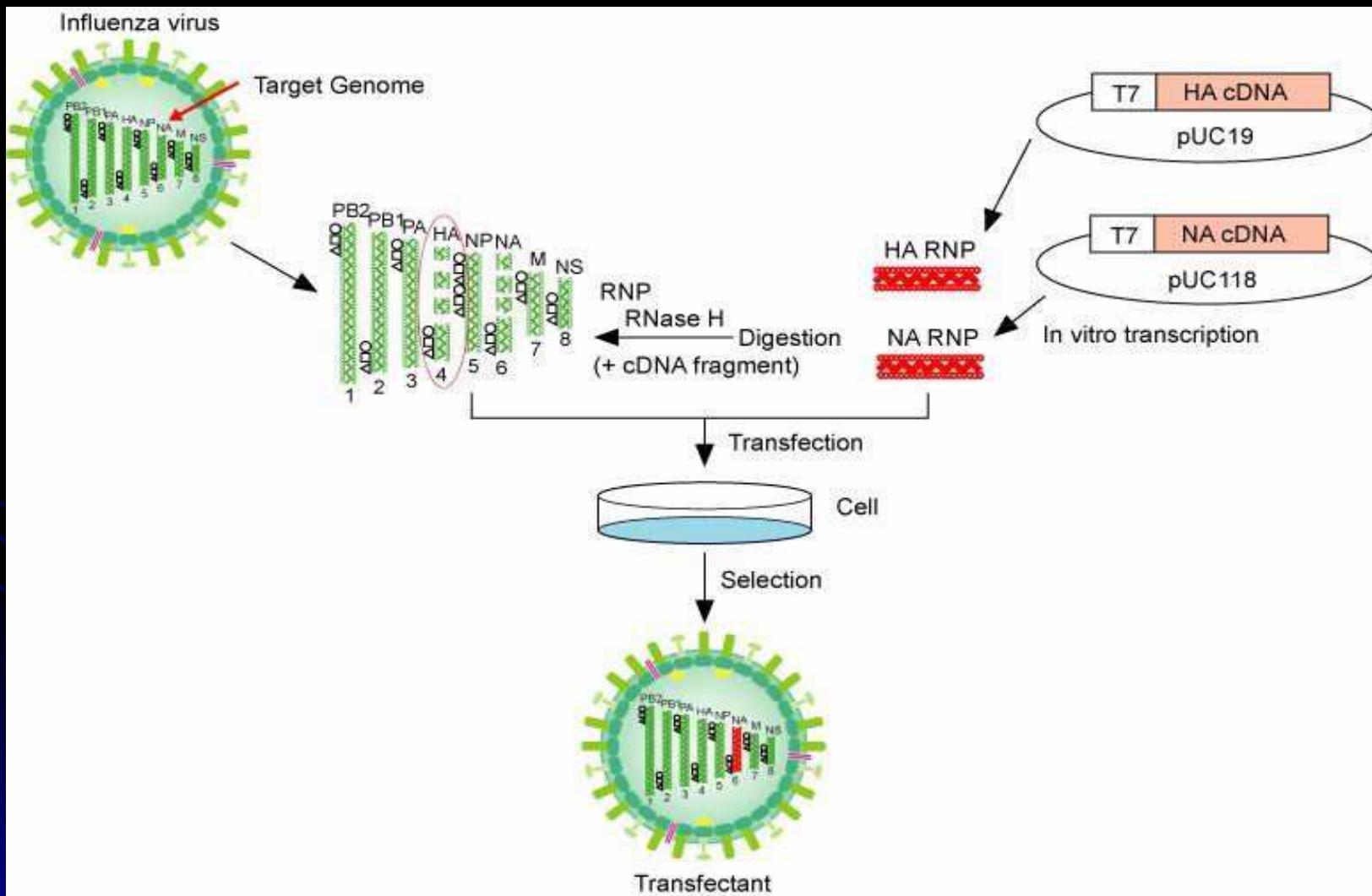
Ilustrasi Pembuatan Master Seed Vaksin dengan Teknologi Reverse Genetics





Aplikasi Teknologi Reverse Genetics dalam Pembuatan Master Seed Vaksin

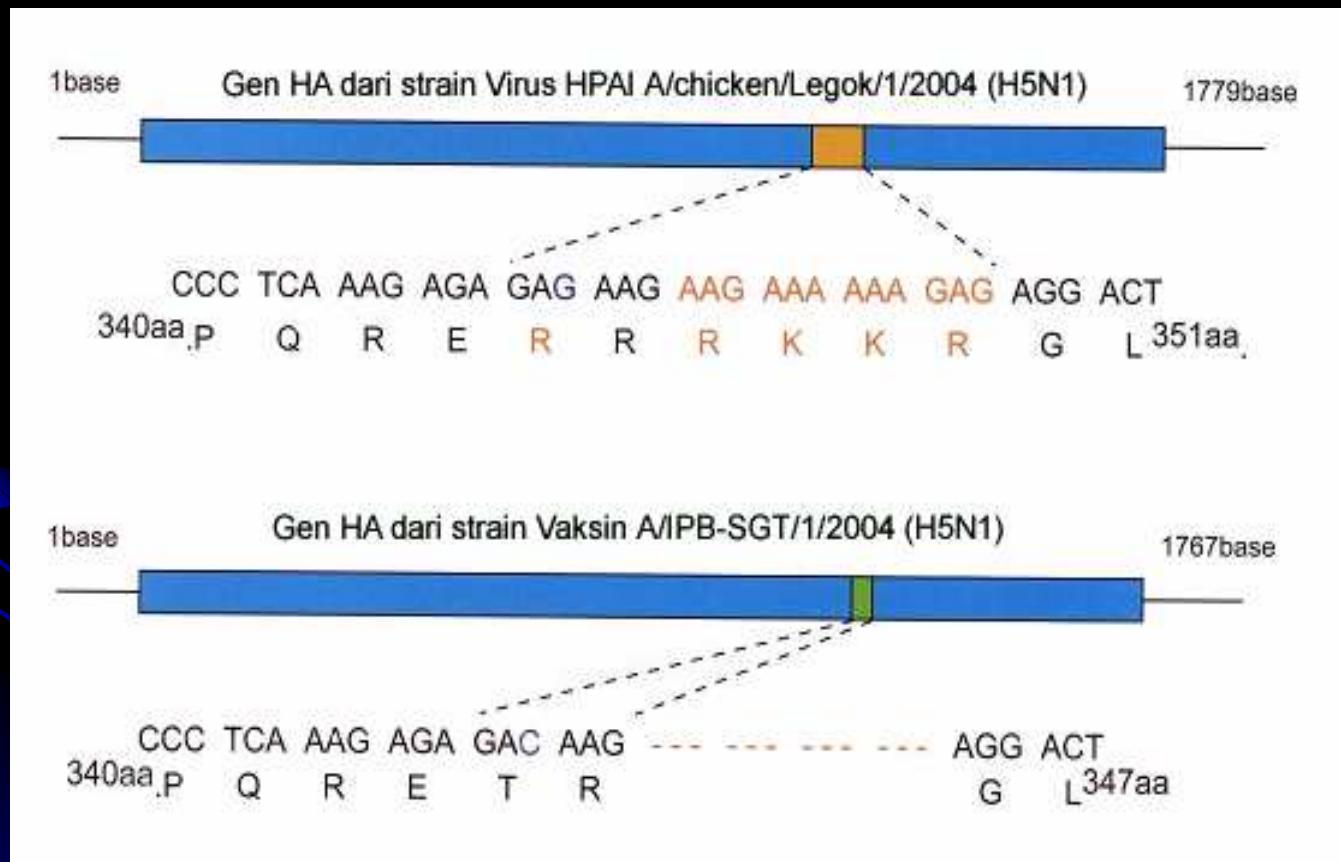
RNP Transfection



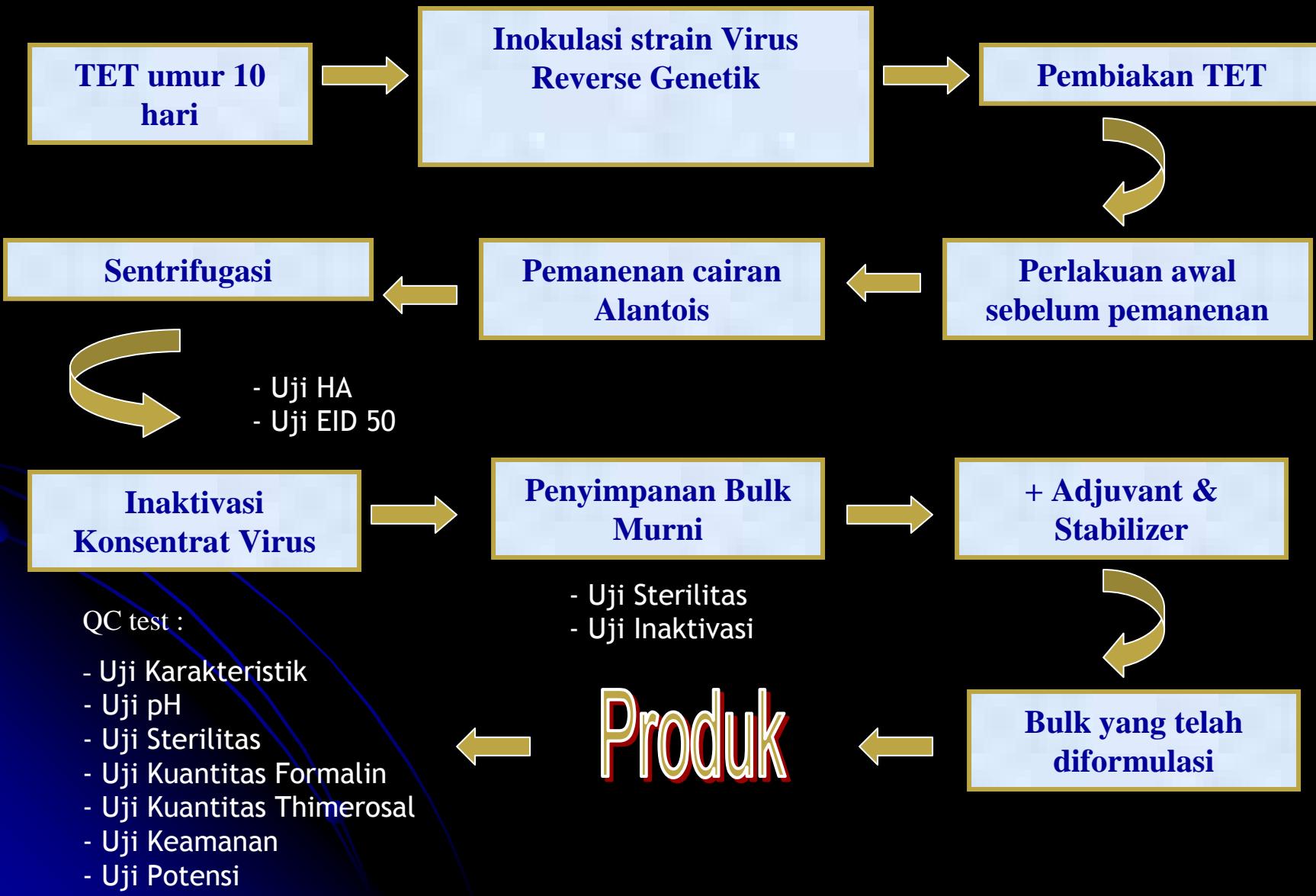
Faktor Virulensi Virus AI

Strain Virus	Sequence Asam Amino
VIRUS VIRULEN	
A/Hong Kong/156/97	R E R R R K K R
A/Hong Kong/213/2003	R E R R R K K R
A/duck/China/E319-2/2003	R E ‘ R R R K R
A/chicken/Shantou/4231/2003	R E R R R K K R
A/chicken/Indonesia/BL/2003	R E R R R K K R
A/chicken/Indonesia/Legok/1/2004	R E R R R K K R
A/Vietnam/1196/2004	R E R R R K K R
A/goose/Thailand/79/2004	R E R R R K K R
A/chicken/Yamaguchi/7/2004	R E ‘ R R K K R
A/chicken/Oita/8/2004	R E ‘ K R K K R
A/chicken/Kyoto/3/2004	R E ‘ R R K K R
VIRUS AVIRULEN	
A/chicken/Mexico/31381/94(H5N2)	R E ‘ ‘ ‘ T R
A/PR/8/34(H1N1)	I Q ‘ ‘ ‘ S R

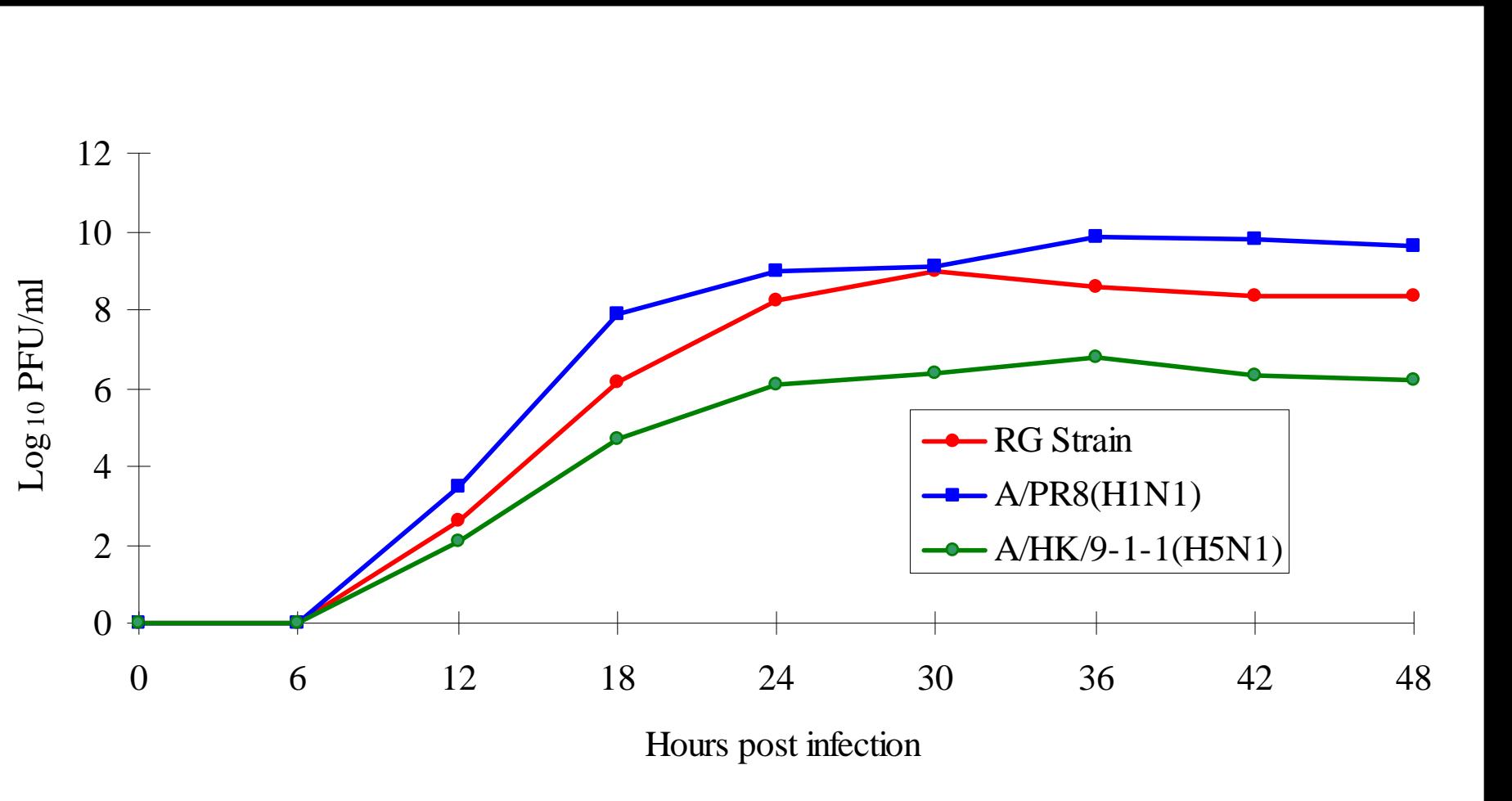
Perbandingan Susunan HA dari RNA dan Asam Amino Antara Virus HPAI dan Strain Vaksin A/IPB-SGT/1/2004



Proses Pembuatan Vaksin Reverse Genetics



Pertumbuhan Virus Reverse Genetics pada Sel MDCK

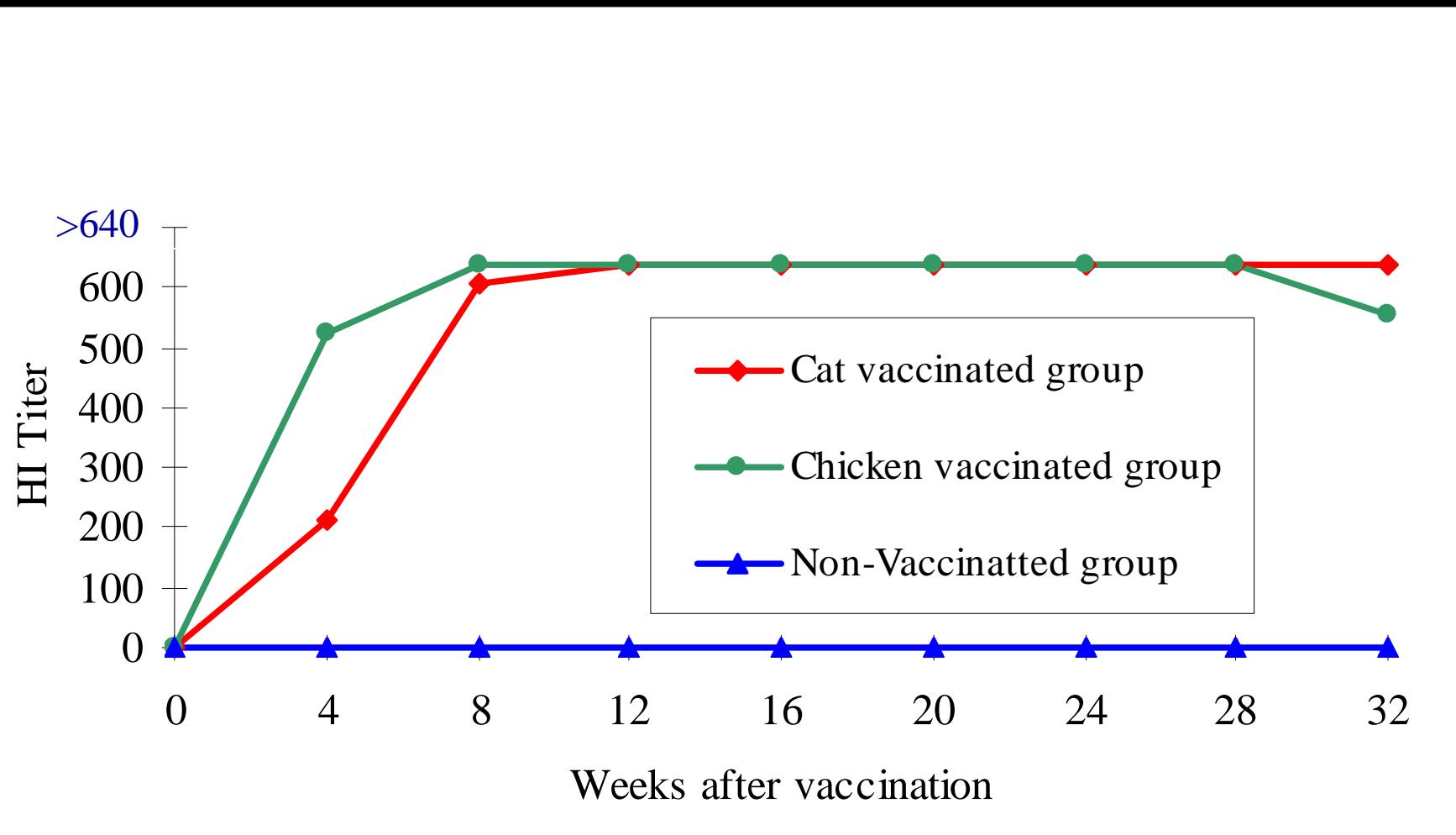


Patogenisitas Vaksin Reverse Genetics pada Ayam

Virus	Asal Gen			MDT(h)	ICPI	IVPI	INPI
	HA	NA	Lainnya				
PR8	PR8	PR8	PR8	72.5	0(0/10)	0(0/10)	0(0/10)
RG Strain	ck/Legok	ck/Legok	PR8	74	0(0/10)	0(0/10)	0(0/10)

MDT Mean Death Time (hour)
ICPI Intra Cerebral Pathogenicity Index
IVPI Intra Venous Pathogenicity Index
INPI Intra Nasal Pathogenicity Index

Level Antibodi pada Serum Kucing dan Ayam setelah Vaksinasi dengan Vaksin Reverse Genetics



Perbandingan Homologi Asam Amino HA Antara Strain H5N1 Legok dengan H5N1 Asia dan H5N2 Amerika/Mexico

Virus	Homologi (%)	Σ asam amino homolog / Σ total asam amino
Indonesia		
A/Dk/Indonesia/MS/2004 (H5N1)	100	564/565
A/Ck/Indonesia/5/2004 (H5N1)	99	558/565
A/Ck/Indonesia/BL/2003 (H5N1)	100	552/553
A/Ck/Indonesia/PA/2003 (H5N1)	100	564/565
A/Ck/Indonesia/2A/2003 (H5N1)	99	560/563
Asia-derived strains		
A/Hong Kong/156/97 (H5N1)	95	540/565
A/Hong Kong/483/97 (H5N1)	93	531/573
A/Hong Kong/486/97 (H5N1)	93	534/573
American/Mexican-derived strains		
A/environment/NY/5626-2/98 (H5N2)	87	313/361
A/duck/NY/44018-2/00 (H5N2)	89	503/568
A/Mallard duck/Pennsylvania/10218/84 (H5N2)	86	505/534
A/Turkey/Virginia/6962/83 (H5N2)	81	280/346
A/Chicken/Virginia/40018/84 (H5N2)	81	280/346
A/Chicken/New Jersey/12508/86 (H5N2)	81	280/346
A/Chicken/Massachusetts/11801/86 (H5N2)	81	279/346
A/Chicken/Ohio/22911-10/86 (H5N2)	81	279/346
A/Chicken/Florida/27716-2/86 (H5N2)	81	280/346
A/Chicken/Washington/13413/84 (H5N2)	80	208/261
A/Ck/Mexico/31381-3/94 (H5N2)	85	286/336

Perbandingan Homologi Asam Amino NA Antara Strain H5N1 Legok dengan H5N1 Asia dan H5N2 Amerika/Mexico

Virus	Homologi (%)	Σ asam amino homolog / Σ total asam amino
Indonesia		
A/Dk/Indonesia/MS/2004 (H5N1)	99	443/449
A/Ck/Indonesia/5/2004 (H5N1)	100	433/435
A/Ck/Indonesia/BL/2003 (H5N1)	99	446/449
A/Ck/Indonesia/PA/2003 (H5N1)	100	447/449
A/Ck/Indonesia/2A/2003 (H5N1)	99	430/435
Asia-derived strains		
A/Hong Kong/156/97 (H5N1)	90	407/450
A/Hong Kong/483/97 (H5N1)	87	405/464
A/Hong Kong/486/97 (H5N1)	88	406/464
American/Mexican-derived strains		
A/environment/NY/5626-2/98 (H5N2)	44	208/476
A/duck/NY/44018-2/00 (H5N2)	43	215/495
A/duck/NJ/117228-7/01 (H5N2)	43	213/495
A/Chicken/Pennsylvania/8125/83 (H5N2)	44	211/476

Perbandingan Homologi Rangkaian Asam Amino HA

Indonesian Epidemic Strain

- A/Ck/Indonesia/Legok/2004(H5N1)
- A/Dk/Indonesia/MS/2004(H5N1)
- A/Ck/Indonesia/5/2004(H5N1)
- A/Ck/Indonesia/BL/2003(H5N1)
- A/CK/Indonesia/PA/2003(H5N1)
- A/Ck/Indonesia/2A/2003(H5N1)

Strain Vaksin unggas yang beredar saat ini

86%

- A/chicken/
Mexico/232/94
(H5N2)

>99%

Virus yang dibuat dgn
Reverse Genetic

PERAN HA DAN NA DALAM INFEKSI

"Whereas the HA protein had the greater effect on the pathogenicity, NA protein also contributes to modulate pathogenicity. Using rescued viruses bearing recombinant HA and NA proteins we showed amino acids in the globular head of HA, in addition to those in the cleavage site, and a glycosylation site in NA play a major role in the pathogenicity (Hulse et al. 2004).

SHEDDING VIRUS

VACCINE	CHALLENGE (10^7 ID50)	HOMOLOGY	TITER	SHEDDING
Homolog	Homolog	rendah	32	14/16, $10^{3.3}$
Homolog	Homolog	tinggi	32	2/16, $10^{1.3}$
A	A'	?	?	?
A	A''	?	?	?

Vaksin Reverse Genetics & Program DIVA (Differentiating Infected from Vaccinated Animals)

Vaccination programs for the control of avian influenza (AI) in poultry have limitations due to the problem of differentiating between vaccinated and virus-infected birds. We have used NS1, the conserved nonstructural protein of influenza A virus, as a differential diagnostic marker for influenza virus infection. Experimentally infected poultry were evaluated for the ability to induce antibodies reactive to NS1 recombinant protein produced in *Escherichia coli* or to chemically synthesized NS1 peptides.

The results demonstrate the potential benefit of a simple, specific ELISA for anti-NS1 antibodies that may have diagnostic value for the poultry industries.

A photograph of a white hen with a red comb and wattle standing behind a group of approximately ten small, fluffy yellow chicks. The hen is facing towards the right of the frame. The chicks are scattered in front of her, some looking towards the camera and others slightly away. The background is a plain, light color.

Sekian & Terima Kasih

13 Mei 2006