

Baxter H5N1 Vaccine

Development and Evaluation

Otfried Kistner

**WHO Meeting on Development and Evaluation of Influenza Pandemic Vaccines
Geneva, November 2 – 3, 2005**

Summary of Baxter's Serum Protein Free Vero Cell Technology

Origin: African Green Monkey (*Cercopithecus aethiops*) kidney cells ATCC CCL81 obtained from ATCC (American Type Culture Collection) at passage no. 124 in 1988.

Cell Banks: MCB (Master Cell Bank) passage no. 128
(fully tested for absence of tumorigenicity, absence of adventitious agents and identity/genetic stability)

WCB (Working Cell Bank) passage no. 133

standard quality control tests:

- bacterial and mycotic sterility
- mycoplasmas
- extraneous agents

Fermentation: Stirred tank fermenter with microcarriers up to 6000 liters

Host System: Influenza viruses (*Orthomyxovirus*)
Hepatitis A virus (*Picornavirus*)
Japanese Encephalitis virus (*Flavivirus*)
West Nile virus (*Flavivirus*)
Vaccinia virus / Smallpox vaccine ACAM 2000 (*Poxvirus*)
SARS Coronavirus (*Coronavirus*)

Bohumil Facility, Czech Republic: A fully GMP Licensed Plant Dedicated to the Production of Cell Culture Influenza Vaccines



Capacity:

Upto 20 Million doses trivalent bulk per year with 15 μg HA per strain

Bohumil Facility, Czech Republic

Fermentation, Purification, and Finalization of Bulk Product



Titers of Different Influenza A Virus Strains of Human or Animal Origin in Serum Protein Free Vero Cell Cultures

Subtype	Host	Strain	Hemagglutinating Units (HAU)
H1N1	Human	<i>A/PR/8/34</i>	256
	Human	<i>A/USSR/90/77</i>	256
	Swine	<i>A/Swine/1976/31</i>	256
	Duck	<i>A/Duck/Bavaria/2/77</i>	256
H2N2	Human	<i>A/Singapore/1/57</i>	128
H3N2	Human	<i>A/Hong Kong/1/68</i>	128
	Swine	<i>A/Swine/Hong Kong/3/76</i>	128
	Swine	<i>A/Swine/Hong Kong/127/82</i>	256
	Duck	<i>A/Duck/Hong Kong/24/75</i>	256
H5N3	Duck	<i>A/Duck/Singapore/3/97</i>	256
H7N1	Fowl	<i>A/FPV/Rostock/34</i>	256
H9N2	Fowl	<i>A/Quail/Hongkong/G1/97</i>	128
	Human	<i>A/Hongkong/1073/99</i>	256
H5N1	Human	<i>A/Hong Kong/213/2003</i>	256
		<i>A/Viet Nam/1203/2004</i>	1024
		<i>A/Viet Nam/1194/2004</i>	1024
		<i>A/SP83/2004 (Thailand)</i>	512

Growth Kinetics of Human Isolates of H5N1 Wildtype Viruses in Serum Protein Free Vero Cell Cultures

Strain	Source	Trypsin	Day 1		Day 2		Day 3	
			HAU	TCID ₅₀	HAU	TCID ₅₀	HAU	TCID ₅₀
A/HK/213/2003	Egg	-	32	7.2	256	8.0	64	7.0
		+	128	7.2	256	6.7	n.d.	n.d.
A/VN/1203/2004	Egg	-	128	7.6	512	8.0	256	7.4
		+	512	7.8	1024	8.4	n.d.	n.d.
A/VN/JP/1203/2004	Cell Culture	-	16	7.6	256	8.5	256	8.0
		+	64	8.1	1024	8.2	n.d.	n.d.

Characteristics of Baxter's Vero Cell-Derived Pandemic-Like H5N1 Candidate Influenza Vaccine

Monovalent: Doses in the range of 3.75 µg – 45 µg of hemagglutinin; non-adjuvanted or adjuvanted with 0.2% aluminium hydroxide

Grown on a qualified continuous cell line (Baxter's serum protein free Vero cells) using **egg-derived wildtype** virus provided by CDC

Double inactivated for enhanced safety affecting two different targets: **protein** by formalin treatment and **viral RNA** by UV irradiation

Sucrose gradient purified **whole virus** vaccine

Free of preservatives and antibiotics

Sterile filtrated and filled in single-use syringes or vials

Immunogenicity of an Inactivated Purified Wildtype H5N1 Viet Nam 1203 Whole Virus Antigen Preparation in Mice and Guinea Pigs

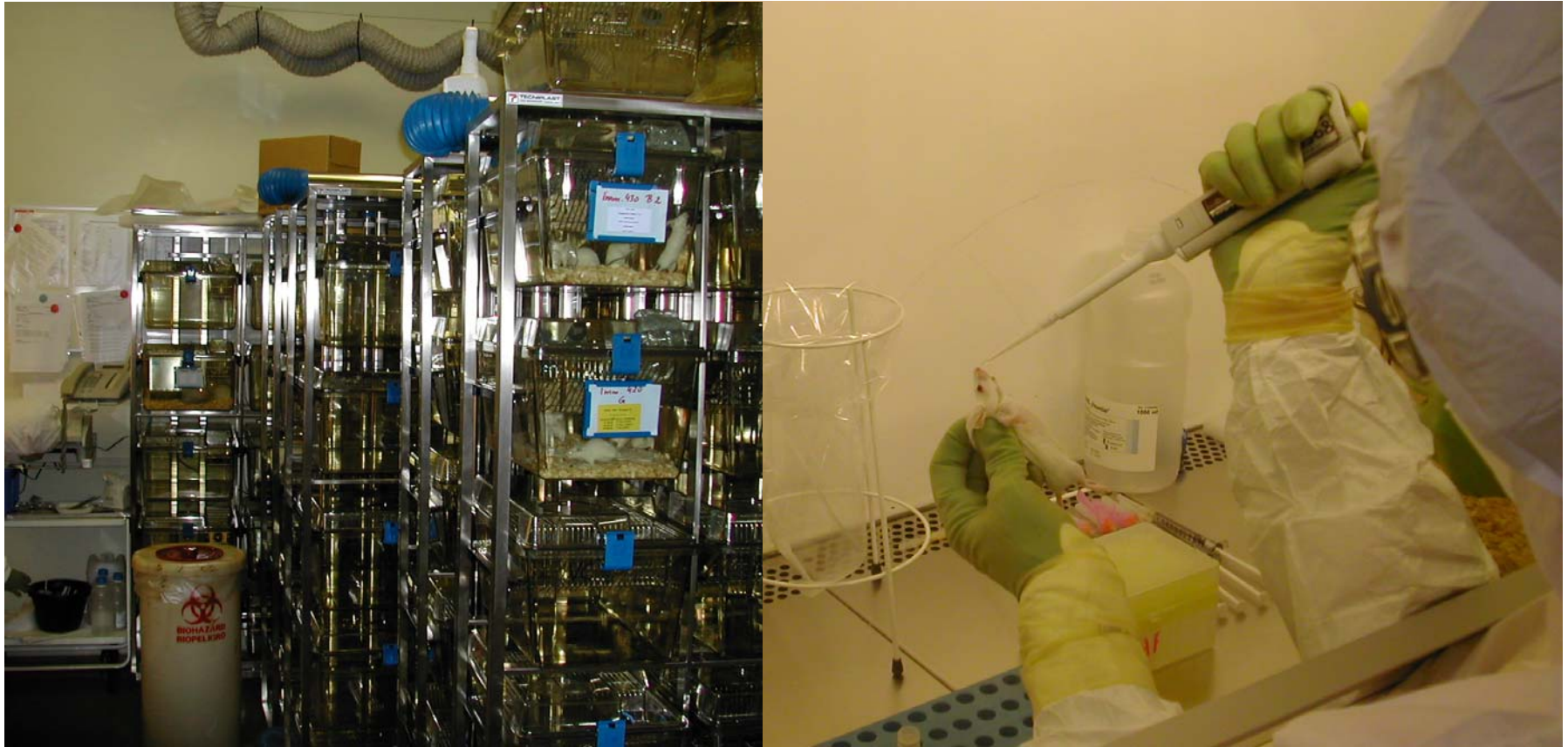
Immunization	anti H5 rHA ELISA Titer		anti H5N1 ELISA Titer		Micro NT Titer		HI Titer	
	Mice	Guinea Pigs	Mice	Guinea Pigs	Mice	Guinea Pigs	Mice	Guinea Pigs
H5N1 Antigen 0.2% Al(OH) ₃ 1.7 µg HA)	11,994	51,200	95,543	162,550	293	4305	13	453
Buffer Control	< 100	< 100	< 100	< 100	≤ 28	≤ 28	5	5

rHA: recombinant Baculo-derived hemagglutinin

NT: neutralisation

HI: hemagglutination

Animal Housing – Challenge of Mice with Infectious H5N1 Virus under Enhanced BSL-3 Conditions



Protection of Mice Against Intranasal Infection with Infectious H5N1 Viet Nam 1203 Virus after 2 Immunizations with an Inactivated Purified H5N1 Viet Nam 1203 Whole Virus Antigen Preparation

Immunization	Mean – anti H5 rHA ELISA Titer	Mean – Micro NT Titer	Surviving animals
H5N1 Antigen 0.2% Al(OH) ₃ 1.7 µg HA	25,600	494	10 / 10
Buffer Control	≤ 100	≤ 7	0 / 10

rHA = recombinant Baculo-derived hemagglutinin

NT = neutralisation titer

➤ Immunization an inactivated purified H5N1 Viet Nam 1203 whole virus antigen preparation (2 doses of 1.7 µg HA each) protected 100% of the immunized mice against intranasal challenge with 10^5 TCID₅₀ (= 2×10^2 LD₅₀) infectious Viet Nam 1203 virus, whereas all control animals died between 4 and 8 days after infection

Immunization of CD1 Mice with Adjuvanted Monovalent Bulk of H5N1 Candidate Vaccine: Survivors after Challenge

Antigen	Dose HA Antigen	Survivors (n/n_{total})	rHA Elisa Titer	HI Titer	NT Titers
MVB #13/07/05 (- trypsin)	3.3 µg	5/5	102,400	40	698
	0.33 µg	4/5	25,600	10	104
	0.03 µg	2/5	528	5	≤ 57
MVB #14/07/05 (+ trypsin)	4.7 µg	5/5	25,600	40	320
	0.47 µg	5/5	33,779	40	349
	0.05 µg	2/5	303	5	≤ 57
Control	-	0/5	< 100	5	≤ 57

rHA: recombinant Baculo derived hemagglutinin

HI: hemagglutination inhibition

NT: neutralisation

Immunogenicity in Mice of Adjuvanted MVB of H5N1 Candidate Vaccine: Effective (ED₅₀) and Protective (PD₅₀) Dose 50%

MVB 13/07/05		MVB 14/07/05	
- Trypsin		+ Trypsin	
ED ₅₀	PD ₅₀	ED ₅₀	PD ₅₀
week 3			
426 ng	n.d.	487 ng	n.d.
week 6			
67 ng	169 ng	67 ng	143 ng

n.d. not determined

Immunisation of Chimpanzees with Egg and Vero-Derived Whole Virus Influenza Vaccines of Seasons 1996/97 and 1997/98

		<i>Texas 36</i> 1996/97	<i>Joh 82</i> 1997/98	<i>Nanchang</i>		<i>B/Harbin</i>	
				1996/97	1997/98	1996/97	1997/98
1. Seroconversion (% with 4-fold and ≥ 40 HI-titer increase)							
Group I	Vero 15µg/strain	85%	100%	77%	92%	85%	100%
Group II	Vero 5µg/strain, Al(OH) ₃	100%	100%	80%	100%	100%	100%
Group III	Vero 1,5µg/strain, Al(OH) ₃	92%	85%	92%	100%	85%	92%
Group IV	Egg 15µg/strain	69%	92%	69%	67%	77%	83%
CPMP criterion		> 40%		> 40%		> 40%	
2.a. GMT titer							
Group I	Vero 15µg/strain	168,8	494,7	84,0	440,6	58,1	143,8
Group II	Vero 5µg/strain, Al(OH) ₃	160,0	710,6	69,6	752,7	46,0	242,5
Group III	Vero 1,5µg/strain, Al(OH) ₃	208,9	431,0	104,4	387,4	64,6	166,6
Group IV	Egg 15µg/strain	160,0	495,9	64,6	84,8	58,1	269,1
2.b. GMT Increase (pre- / post vaccination)							
Group I	Vero 15µg/strain	8,0	34,8	8,4	11,6	8,0	8,6
Group II	Vero 5µg/strain, Al(OH) ₃	9,2	53,8	7,0	30,2	7,0	29,6
Group III	Vero 1,5µg/strain, Al(OH) ₃	11,6	18,3	9,9	11,0	8,9	10,1
Group IV	Egg 15µg/strain	5,5	24,6	4,9	8,5	6,8	15,0
CPMP criterion		> 2,5		> 2,5		> 2,5	
3. Protective Titer (% with HI titer ≥ 40)							
Group I	Vero 15µg/strain	100%	100%	92%	92%	100%	100%
Group II	Vero 5µg/strain, Al(OH) ₃	100%	100%	100%	100%	100%	100%
Group III	Vero 1,5µg/strain, Al(OH) ₃	100%	100%	100%	100%	100%	100%
Group IV	Egg 15µg/strain	100%	92%	100%	100%	92%	92%
CPMP criterion		> 70%		> 70%		> 70%	

Induction of CMI (Cell Mediated Immunity) Determined as SI (Stimulation Index) in Mice by Different Influenza Vaccines

	A/Johannesburg/82/96 (A/H1N1)		A/Nanchang/933/95 (A/H3N2)		B/Harbin/7/94 (B)		Vero cell / egg proteins	
	Vero-Ag	Egg-Ag	Vero-Ag	Egg-Ag	Vero-Ag	Egg-Ag	Vero-Ag	Egg-Ag
Vero whole virus vaccine	29.1 ± 6.2	13.1 ± 3.2	52.3 ± 9.2	21.4 ± 3.2	15.1 ± 3.3	13.4 ± 3.7	0.9 ± 0.2	1.4 ± 0.3
Egg whole virus vaccine	9.0 ± 2.1	13.0 ± 3.1	34.4 ± 7.1	20.5 ± 3.1	7.2 ± 1.4	9.1 ± 1.1	2.0 ± 0.8	1.9 ± 0.6
Egg split virus vaccine	13.3 ± 2.4	5.2 ± 0.2	23.4 ± 2.3	16.1 ± 2.0	5.3 ± 0.8	6.3 ± 0.2	1.6 ± 0.6	0.7 ± 0.2
Egg subunit virus vaccine	1.6 ± 0.3	1.2 ± 0.2	2.5 ± 0.3	1.1 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Vero mock preparation	1.2 ± 0.1	1.6 ± 0.4	2.0 ± 0.3	1.2 ± 0.3	1.6 ± 0.2	0.7 ± 0.2	1.3 ± 0.1	1.5 ± 0.3
Egg mock preparation	0.4 ± 0.2	2.7 ± 0.7	0.8 ± 0.3	1.7 ± 0.3	0.4 ± 0.1	1.7 ± 0.1	0.5 ± 0.2	0.6 ± 0.2

H5N1 Candidate Vaccine Development Program

- **Development of a safe and immunogenic inactivated purified Vero cell derived whole virus H5N1 candidate vaccine and production of GMP clinical grade material using H5N1 wildtype virus A/Viet Nam/1203/2004**
 - Establishment & testing of viral banks – completed
 - Process development – completed
 - Pre-clinical testing – in progress
 - Formulation, fill & finish of the vaccine – beginning 2006
- **Planned formulations**
 - 3,75 µg + adjuvant
 - 7,5 µg +/- adjuvant
 - 15 µg +/- adjuvant
 - 45 µg - adjuvant
- **Clinical Studies**
- **Filing of EU Mock-up Dossier and US IND**

NIH Contract

Baxter is working with the National Institute of Allergy and Infectious Diseases, part of the National Institutes of Health, to develop a cell culture-based (Vero) H5N1 candidate pandemic influenza vaccine. Baxter will be providing the candidate vaccine to NIAID for clinical testing. Clinical testing is expected to be initiated in 2006

Advantages of Baxter's Vero Cell Derived Pandemic Vaccine Production

- So far all strains of human and animal origin (H1, H2, H3, H5, H7, H9) tested show high and consistent growth; with even significantly higher growth of the recent H5N1 human wild type isolates
- No supply issue with eggs; Vero cell production can be started at any time and on a continuous basis
- All plants (Orth, Bohumil) designed for BSL (Biosafety Level) 3
- No need for High Growth Reassortant or attenuated reverse genetic strain(s) for production; thus allowing start of vaccine production directly after receipt of wild type strain with the first batch available in **10 – 12 weeks**
- The Vero cell technology involves the production of a whole virus vaccine which should be more effective as a pandemic vaccine than split or subunit vaccines in an unprimed population