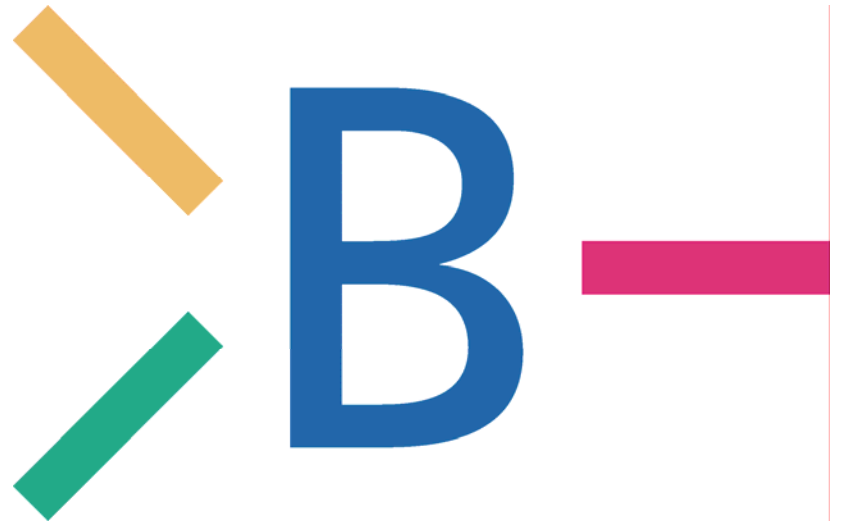


# Berna Biotech H9N2 vaccine: development, pre-clinical and clinical trials

**Dr. Reinhard Glück**

Chief Scientific Officer Berna Biotech

Geneva 2-3 November 2005



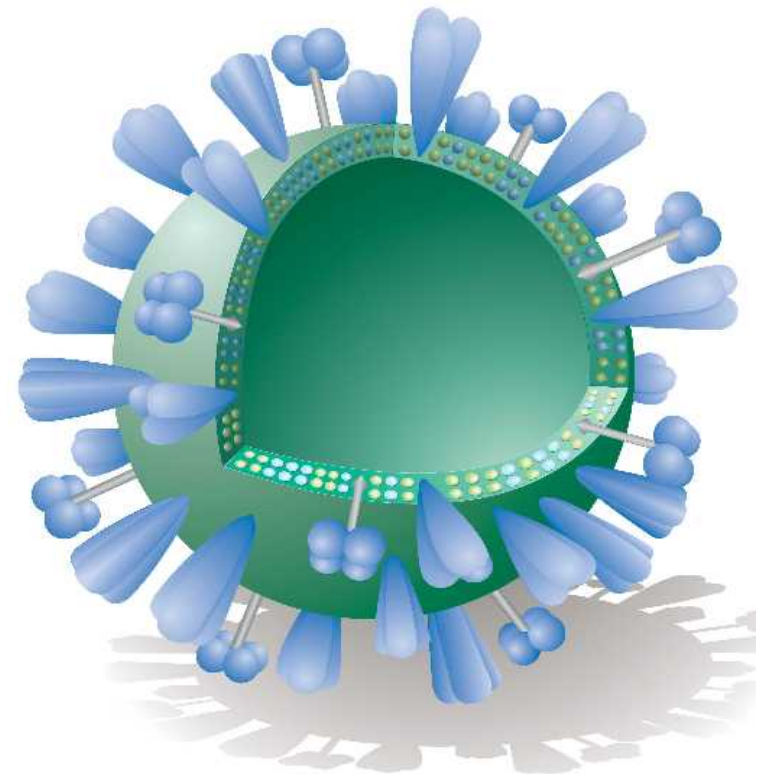
# Influenza vaccines produced by Berna Biotech

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- Whole Virus Vaccine
- Virosome Vaccine
- Intranasal Virosome Vaccine with Mucosal Adjuvant

# Intranasal virosome vaccine



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Production of pandemic vaccine  
can only occur once the exact  
candidate strain is determined

Development work must be done in the  
**interpandemic period**

- Evaluation of the best method of preparation
- Evaluation of the most suitable adjuvant
- Evaluation of the application form
- Evaluation of serological assessment in immunologically naive populations

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Development and evaluation of  
H9N2 influenza pandemic  
vaccines at Berna Biotech and  
Etna Biotech

# Vaccine development

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## Past:

- Protective efficacy of subunit and virosome vaccines in mice (in collaboration with NIBSC)
- Comparison of i.m. and i.n. application in ferrets (challenge)
- Safety and antigenicity of whole virus and subunit influenza vaccines in healthy adults (in collaboration with Leicester Royal infirmary, Solvay, Central Public Health Laboratory Colindale)

# Vaccine development

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## Present:

- Clinical evaluation of adjuvanted and non adjuvanted vaccines (in collaboration with institutions in the UK)

# Protective efficacy of subunit and virosomal H9N2 vaccine in mice

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- 3 groups of 20 mice
- 2 doses of 15  $\mu$ g HA of:
  - conventional subunit (i.m.)
  - virosomal (i.m.)
  - virosomal + mucosal adjuvant (i.n.)
  - saline (i.m. and i.n.)
  - Hemagglutination Inhibition and ELISA
  - Evaluation of IgG, IgG1, IgG2, IgA
  - Challenge with 50 MID<sub>50</sub> of A/HK/1073/99 virus

# Results

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## ELISA activity

- IgG1 responses were highest in mice immunized i.m. with virosome vaccine
- IgG2 responses were highest in mice immunized i.n. with virosome vaccine
- IgA was only detected in nasal washings after i.n. application of virosome vaccine
- Virosome vaccine shifts the balance of the T-helper response in favour of type 1 , i.n. application enhance this shift

# Results

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## HI activity

- Single dose i.m. of virosome vaccine was superior to subunit vaccine
- Two doses i.m. of virosome vaccine was superior to subunit vaccine

## Protection

- I.m. virosome vaccine was more protective than subunit vaccine
- The only vaccine providing complete protection was i.n. applied virosome vaccine



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Vaccine 22 (2004) 4390–4396

Vaccine

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## Strong local and systemic protective immunity induced in the ferret model by an intranasal virosome-formulated influenza subunit vaccine

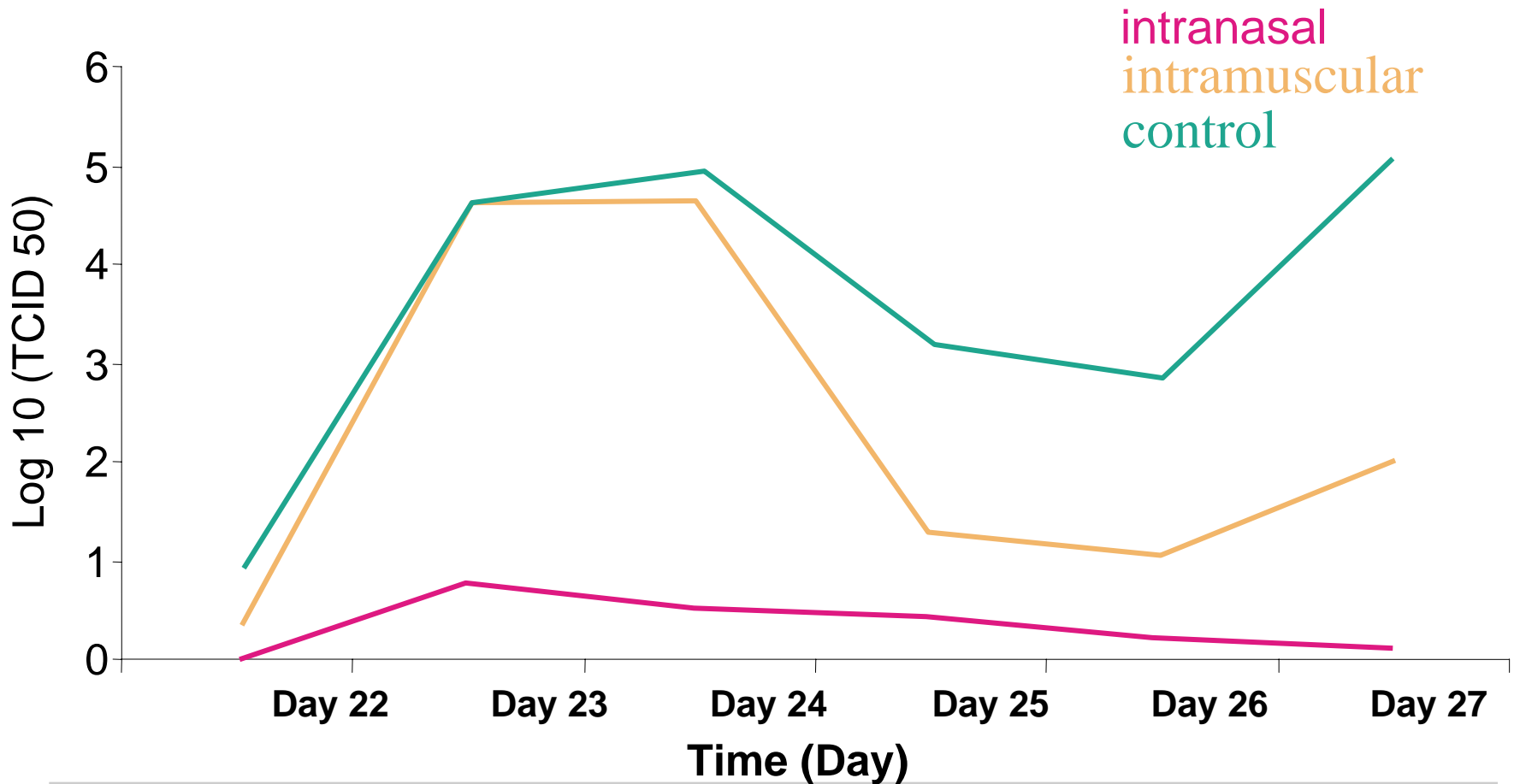
Rob Lambkin<sup>a</sup>, John S. Oxford<sup>a</sup>, Seb Bossuyt<sup>a</sup>, Alex Mann<sup>a</sup>, Ian C. Metcalfe<sup>b</sup>,  
Christian Herzog<sup>b</sup>, Jean-François Viret<sup>b</sup>, Reinhard Glück<sup>b,\*</sup>

<sup>a</sup> *Department of Medical Microbiology and Retroscreen Virology, St. Bartholomew's and the Royal London, Queen Mary School of Medicine and Dentistry, University of London, 327 Mile End Road, London E1 4NS, UK*

<sup>b</sup> *Berna Biotech Ltd., Rehhagstr. 79, CH-3018 Berne, Switzerland*

Received 4 July 2003; accepted 14 October 2003

# Post infective mean viral titres obtained from ferret nasal washes



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## **Safety and antigenicity of whole virus and subunit influenza A/Hong Kong/1073/99 (H9N2) vaccine in healthy adults: phase I randomised trial**

*Iain Stephenson, Karl G Nicholson, Reinhardt Glück, Robert Mischler, Robert W Newman, Abraham M Palache, Neville Q Verlander, Fiona Warburton, John M Wood, Maria C Zambon*

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THE LANCET • Vol 362 • December 13, 2003 • [www.thelancet.com](http://www.thelancet.com)

# Stephenson et al.:

## aim and methods

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- Aim: to assess safety, tolerability and antigenicity of WV and SU H9N2 vaccines in 60 healthy volunteers
- Methods: 2 doses of vaccine with different HA contents (7.5, 15, 30  $\mu\text{g}$ ), given 3 weeks apart
- Primary outcome: antibody response measured by HI and neutralisation test, geometric mean antibody titers 21 days after vaccination

# Stephenson et al.: findings

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Surprisingly 24 out of 60 volunteers had age-related detectable baseline antibody to H9N2 by microneutralisation and hemagglutination inhibition

Only individuals born before 1969

Structural and phylogenetic similarities of the H9 and H2 hemagglutinin could provide an explanation

# Stephenson et al.: findings

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- In participants older than 32 years one dose of either vaccine evoked antibody responses associated with protection
- In participants aged 32 years or younger, antibody responses to one or two doses of whole virus or subunit vaccine were poor, fulfilling none of the EU criteria
- Whole virus vaccine produced a significantly higher probability of seroconversion compared with subunit for this age group

# Stephenson et al.: findings



	<b>≤32 years</b>		<b>&gt;32 years</b>	
	Whole virus (n=14)	Subunit (n=14)	Whole virus (n=12)	Subunit (n=16)
<b>Geometric mean titre increase</b>				
21 days	2.3 (1.4–3.6)	1.7 (0.8–3.3)	2.2 (1.5–3.2)	5.4 (3.0–9.6)
42 days	6.9 (4.6–10.5)	2.8 (1.5–5.4)	3.0 (2.1–4.3)	4.7 (2.7–8.4)
<b>Postvaccination titre &gt;1 in 40</b>				
0	0 (0%)	0 (0%)	2 (17%)	4 (25%)
21 days	3 (21%)	2 (14%)	6 (50%)	12 (75%)
42 days	6 (43%)	2 (14%)	8 (66%)	12 (75%)
<b>Seroconversions</b>				
21	5 (36%)	2 (14%)	6 (50%)	9 (56%)
42	9 (64%)	5 (36%)	9 (75%)	9 (56%)

Data are geometric mean (95% CI) or number of participants (%). CPMP criteria are (for people aged 18–60 years): mean geometric titre increase >2.5; proportion of individuals with post-vaccination titre greater than or equal to 1 in 40 >70%; and percentage of seroconversions >40%.

**Table 5: Haemagglutinin-inhibition results for A/Hong Kong/1073/99 (H9N2) in relation to European Committee of Proprietary Medicinal Products (CPMP) criteria**

# Clinical plan (2005): pandemic trial with H9N2

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- Design:** randomized, observer-blind, dose-ranging, single-centre
- Objectives:** To evaluate the safety, tolerability and immunogenicity of adjuvanted and non-adjuvanted influenza A/Hong Kong/1073/99 (H9N2) vaccines, containing four ascending concentrations of H9 haemagglutinin
- Country:** UK, University of Leicester  
Professor K.G. Nicholson
- Subjects:** Healthy immunologically-naive, adult subjects *N*: 560  
Age: >18
- Virus:** Influenza A/Hong Kong/1073/99

# Primary objectives

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To evaluate the immunogenicity of:

- Non-adjuvanted whole virus vaccine (WV)
- Alum-adjuvanted whole virus vaccine (WV-AI)
- Virosome vaccine (V)
- Whole virus vaccine by intradermal administration

## Secondary objectives

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- To determine the HA content of influenza A/HK/1073/99 (H9N2) WV, WV-AI and V vaccines required to produce a protective antibody response in immunologically naive adult human populations
- To identify whether one or two doses of vaccine are required to satisfy the licensing criteria
- To identify whether i.d. administration is more immunogenic than i.m.
- To examine the short term reactogenicity of the selected vaccines
- To evaluate vaccine type and dose interactions in relation to the age of the patient

# Randomisation and treatment



	Route of administration	No of subjects per group at each dose of HA ( $\mu\text{g}$ )				Total
		<b>1.7</b>	<b>5</b>	<b>15</b>	<b>45</b>	
WV	im	40	40	40	40	160
AI-WV	im	40	40	40	40	160
V	im	40	40	40	40	160
WV	id	40	40			80
<b>Total</b>		160	160	120	120	560

WV: whole virus, AI-WV: whole virus-alum, V: virosome

# Stephenson et al.: findings

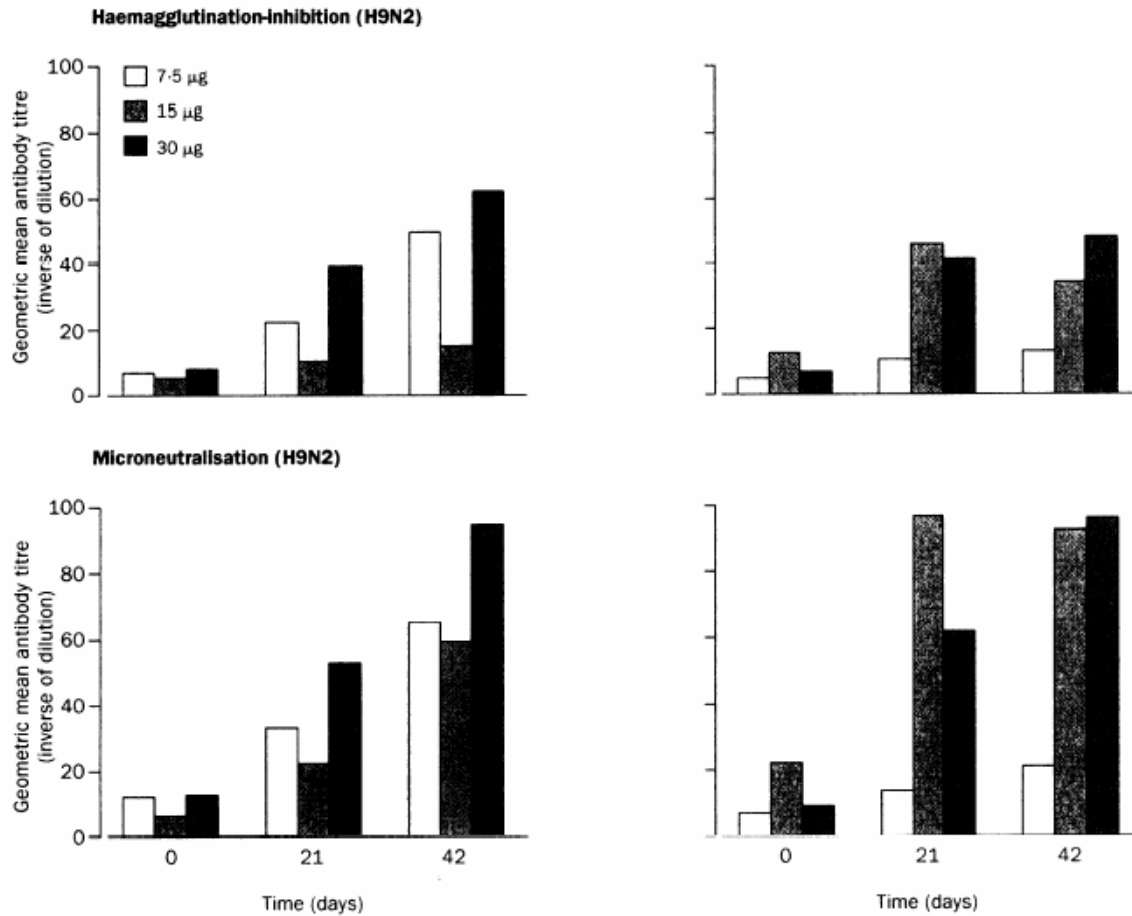


Figure 3: Geometric mean antibody titres to whole virus (left) and subunit (right) vaccine

# Stephenson et al.: findings

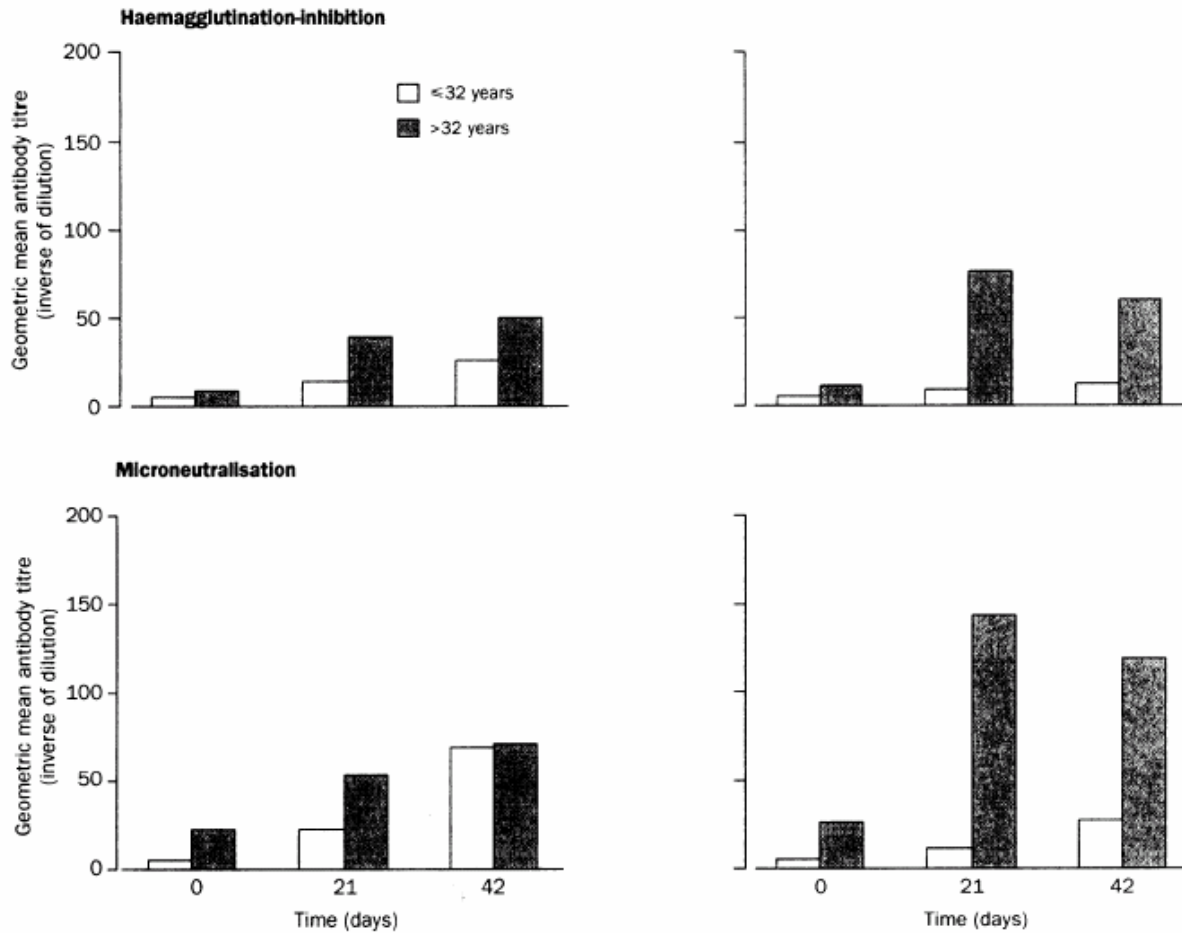


Figure 4: Geometric mean antibody titres to whole virus (left) and subunit (right) vaccine according to age

# Stephenson et al.: findings



	Haemagglutinin content and type of vaccine						p			
	7.5 µg		15 µg		30 µg		Dose	Vaccine type	Age*	Sex
	Whole virus	Subunit	Whole virus	Subunit	Whole virus	Subunit				
<b>Haemagglutination-inhibition</b>										
Day 21	5/10	2/10	2/9	3/10	4/7	6/7	0.222	0.535	0.032	0.923
Day 42	8/10	4/10	4/9	3/10	6/7	7/7	0.048	0.044	0.33	0.992
<b>Microneutralisation ≤32 years</b>										
Day 21	2/4	0/6	0/2	1/4	3/3	1/4	0.103	0.013	*	0.248
Day 42	4/4	2/6	1/2	2/4	3/3	4/4	0.168	0.039	*	0.277
<b>Microneutralisation &gt;32 years</b>										
Day 21	2/6	2/4	3/7	2/6	1/4	6/6	0.102	0.020	*	0.735
Day 42	2/6	2/4	6/7	2/6	2/4	6/6	0.021	0.067	*	0.764

Data are number of seroconversions/number of participants. \*For HI age-vaccine interaction not significant at either day 21 or 42 ( $p > 0.1$ ); for MN age-vaccine interaction significant ( $p = 0.001$  for day 21 and  $p = 0.007$  for day 42).

Table 4: Seroconversions on day 21 and day 42