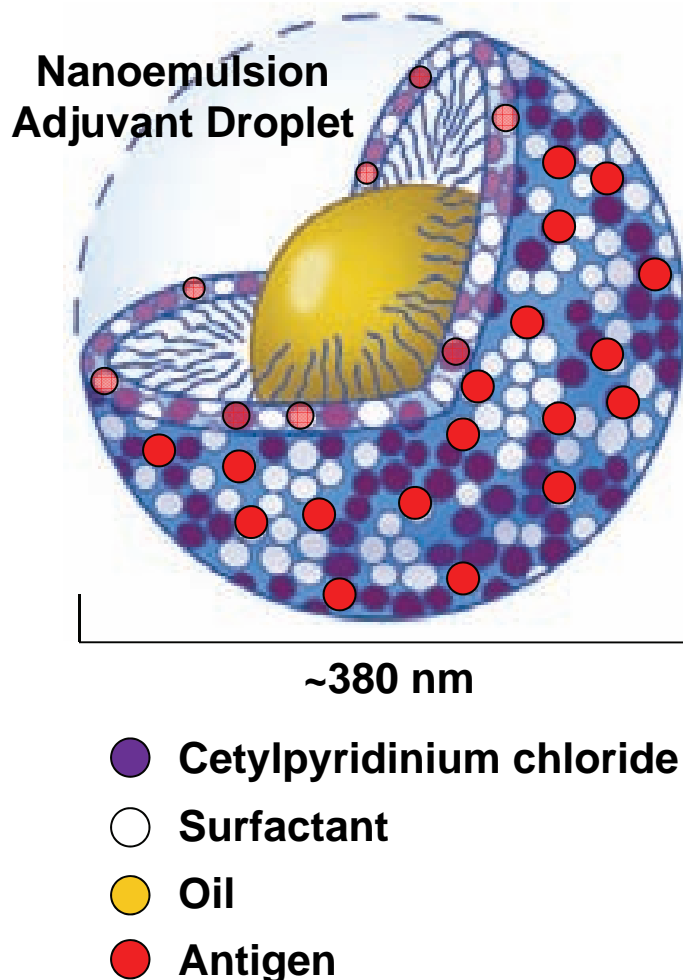


W₈₀5EC, a Novel Nanoemulsion Adjuvant, Enhances the Immune Response to Commercial Influenza Vaccines Following Nasal Administration in Ferrets

Tarek Hamouda, MD, PhD, MBA
Director of Vaccines
NanoBio[®] Corporation

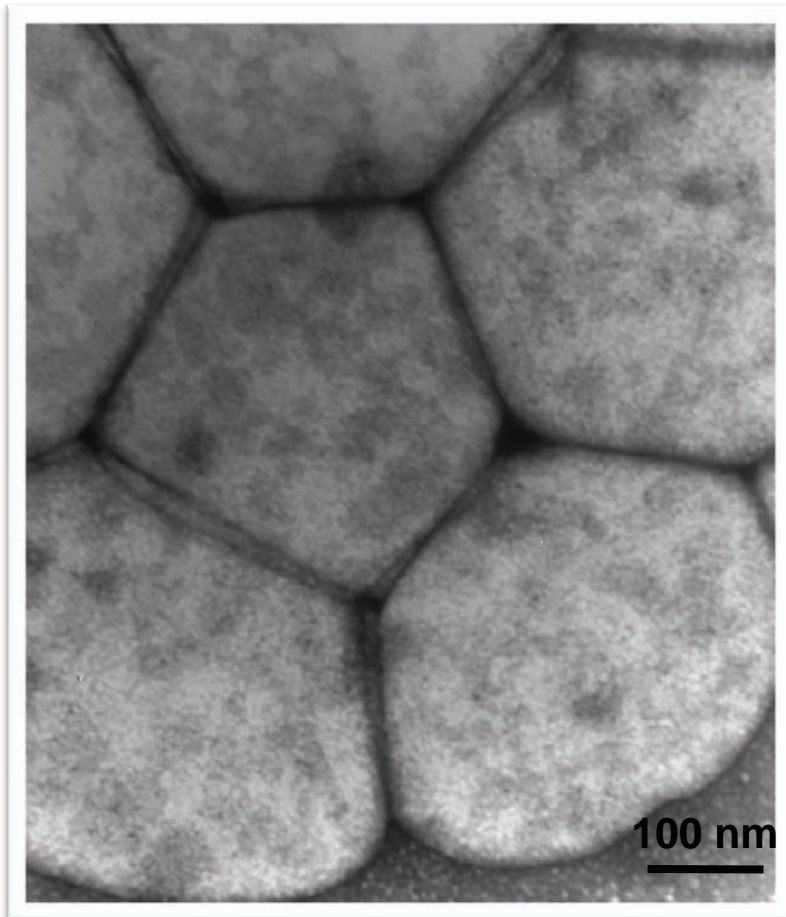
Nanoemulsion (NE) Intranasal Adjuvant Platform



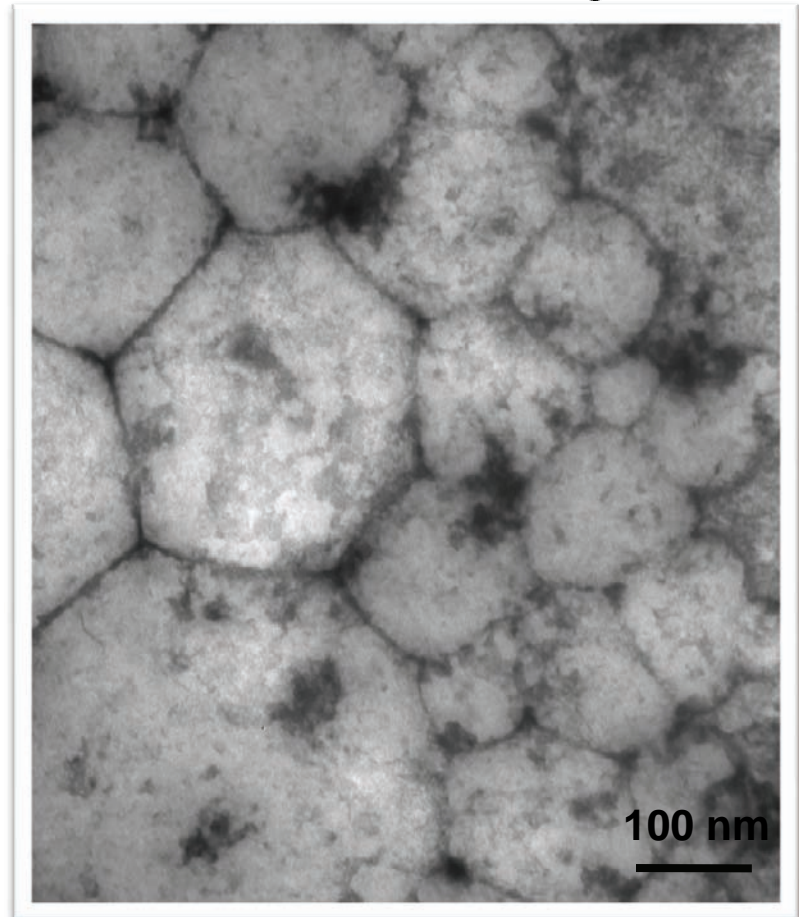
- **Universal** adjuvant platform
- **Significant advantages:**
 - Robust systemic & mucosal immunity
 - Antigen-sparing
 - Cross protection
 - Adjuvants multiple antigen types
 - Non-inflammatory
 - Needle-free
 - Thermally stable
 - Potential to convert live virus to highly effective inactivated vaccines

NE Adjuvanted Influenza Antigen

Nanoemulsion Adjuvant



**Nanoemulsion Adjuvant Mixed
With Influenza Antigen**



Clinical

- Nasal Adjuvanted Seasonal Influenza Vaccine
 - Phase I in 199 subjects

Preclinical

- Hepatitis B*
- RSV
- HIV*
- *S. pneumoniae*
- Pandemic influenza
- Anthrax*
- Small Pox*

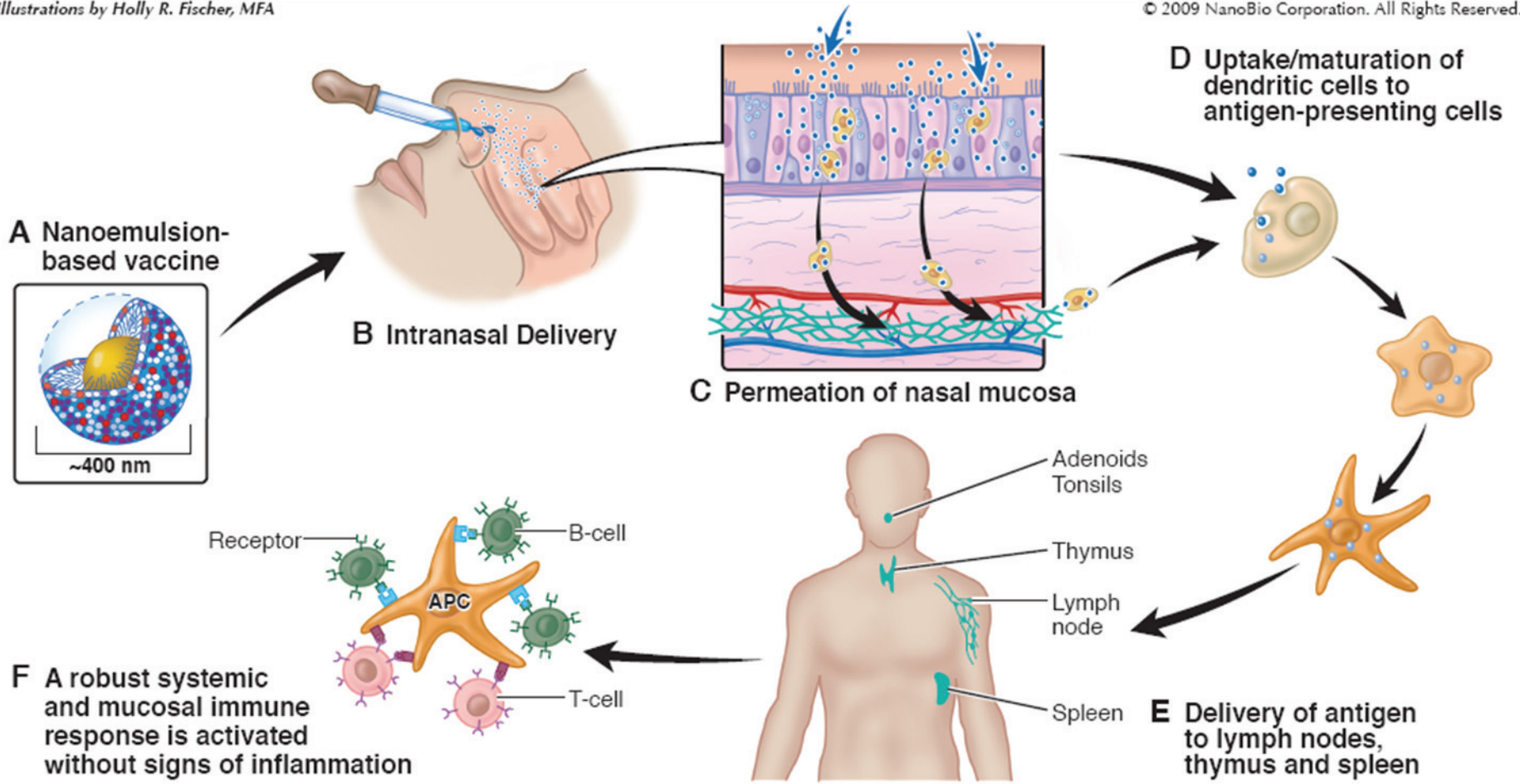
** Data is Published*

How Does it Work?

MECHANISM OF ACTION FOR NANOEMULSION-BASED VACCINES

Illustrations by Holly R. Fischer, MFA

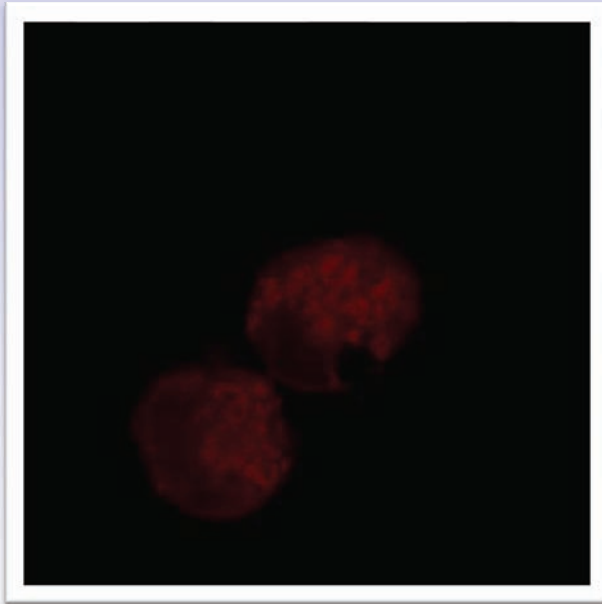
© 2009 NanoBio Corporation. All Rights Reserved.



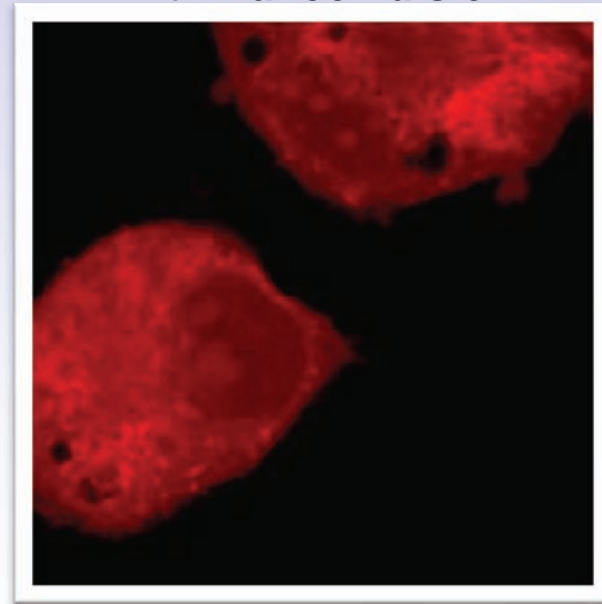
Nanoemulsion Specifically Taken Up By Dendritic Cells

Lipid Content in JawsII Dendritic Cell Line (Red Nile Stain)

Without Nanoemulsion



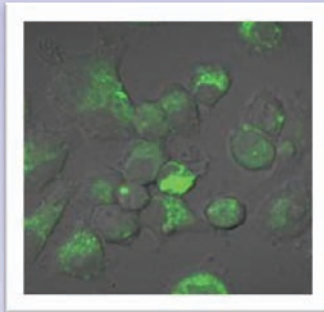
With Nanoemulsion



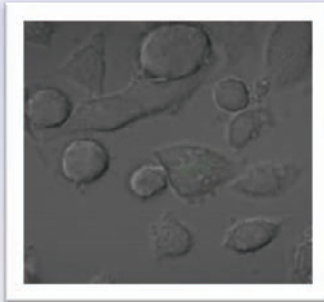
Nanoemulsion Enhances Antigen Internalization into Dendritic Cells

Recombinant Protective Antigen of Anthrax (rPA)

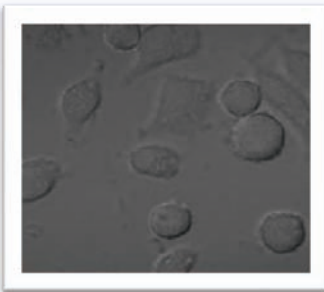
rPA-FITC / NE



rPA-FITC



Control



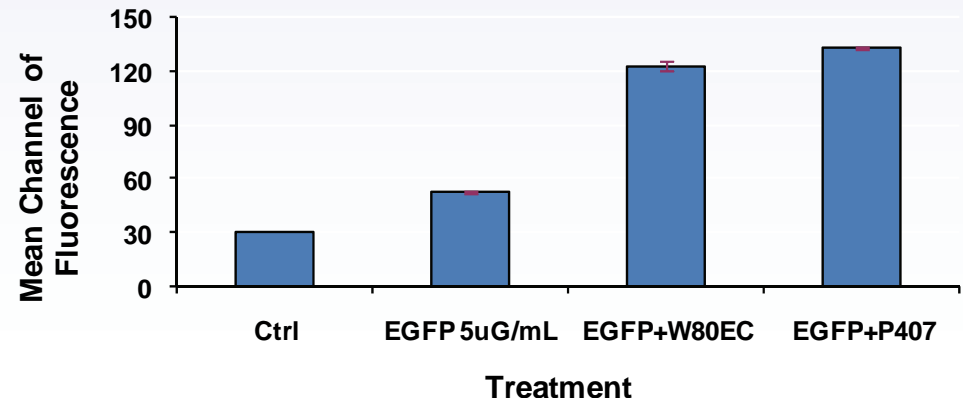
HBsAg Uptake – Western Blot



Legend

1. Cell extract
2. HBsAg only
3. HBsAg-W₈₀5EC 0.001%
4. HBsAg-W₈₀5EC 0.005%
5. HBsAg-P₄₀₇5EC 0.001%
6. HBsAg-P₄₀₇5EC 0.005%

EGFP Uptake - FACS Analysis



Nanoemulsion Enhances Delivery to Immune System

QDOT Without Nanoemulsion

QDOT With Nanoemulsion

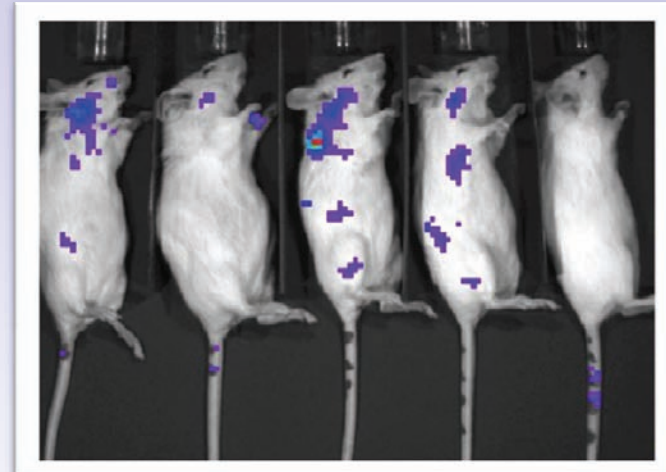
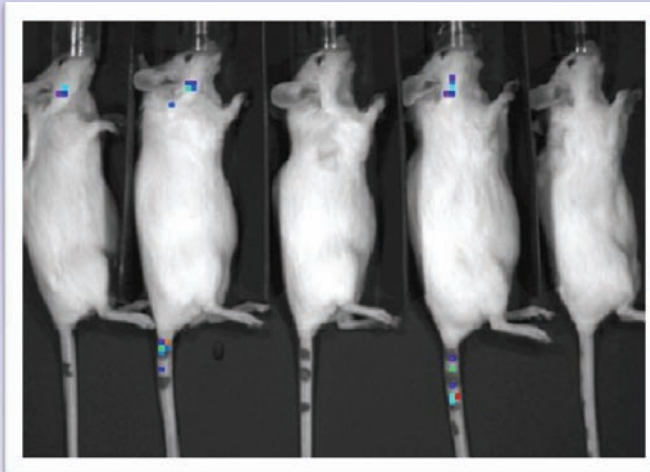
QDOT + PBS

Naive

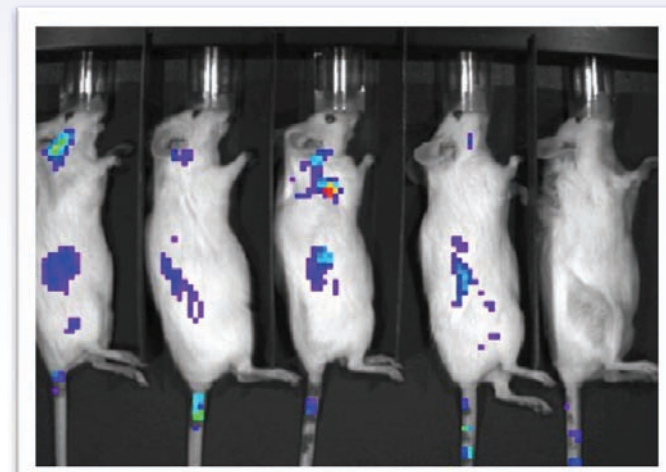
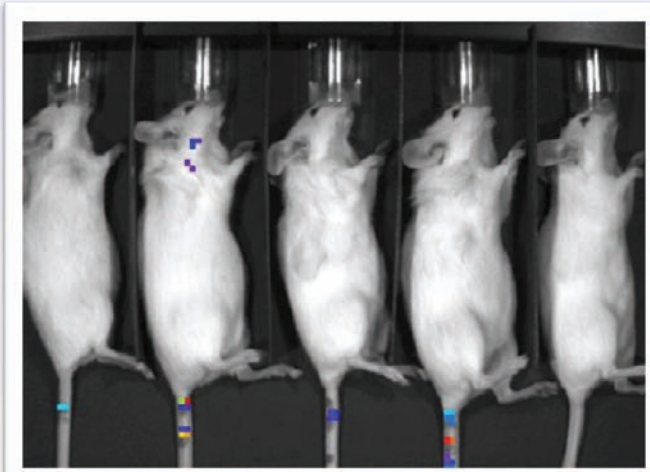
QDOT + NE

Naive

24 Hours



72 Hours



Nanoemulsion Enhances Delivery to Immune System

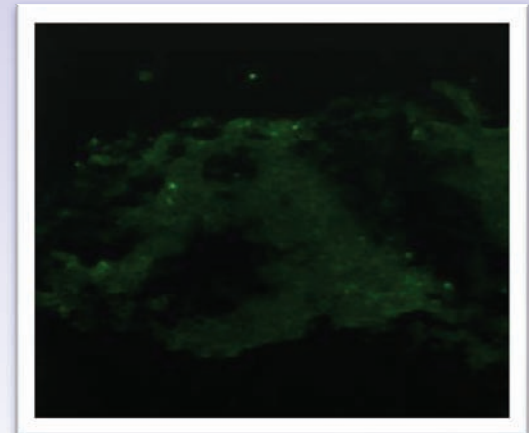
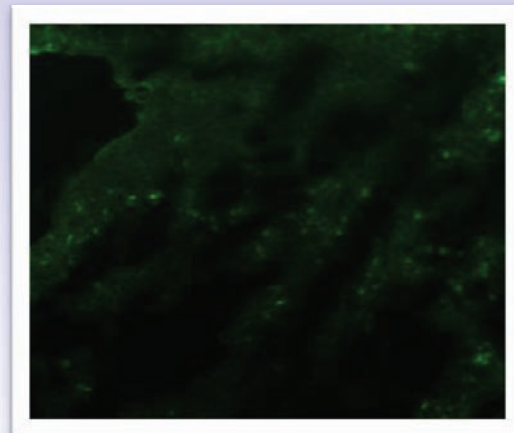
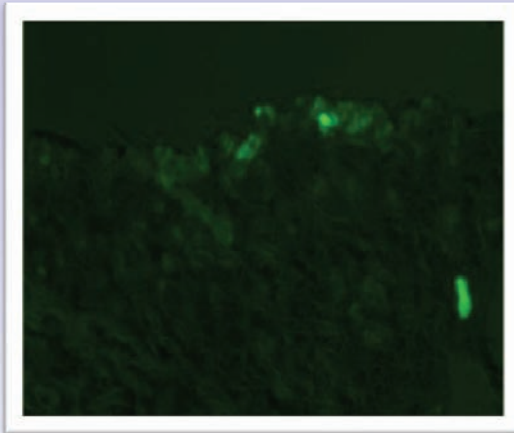
After 24 Hours

Nasal Epithelium

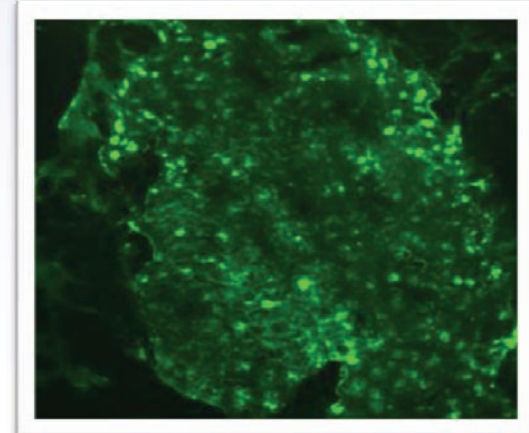
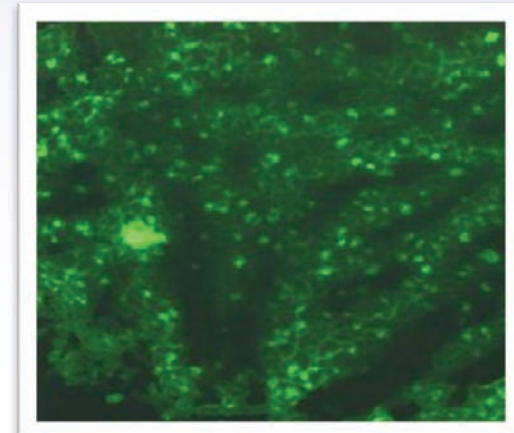
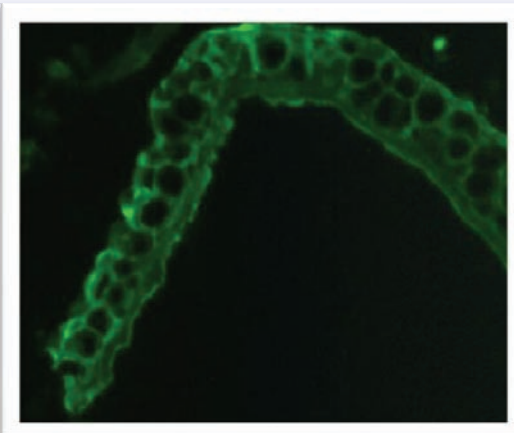
Submandibular Lymph Nodes

Thymus

GFP Alone



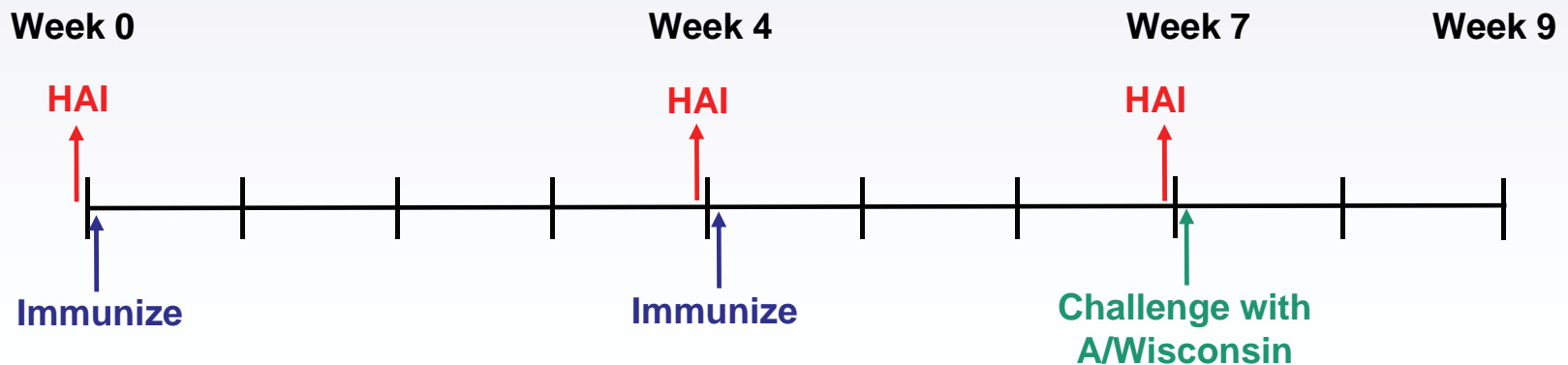
GFP With NE



Nanoemulsion Adjuvanted- Seasonal Influenza Vaccine

- **Purpose:**

- Demonstrate immune response with influenza commercial vaccine adjuvanted with nanoemulsion (NE)
- Assess intranasal administration
- Demonstrate the dose sparing effect of the NE adjuvant
- Demonstrate cross protection to other influenza strains
- Demonstrate protection against challenge with live virus

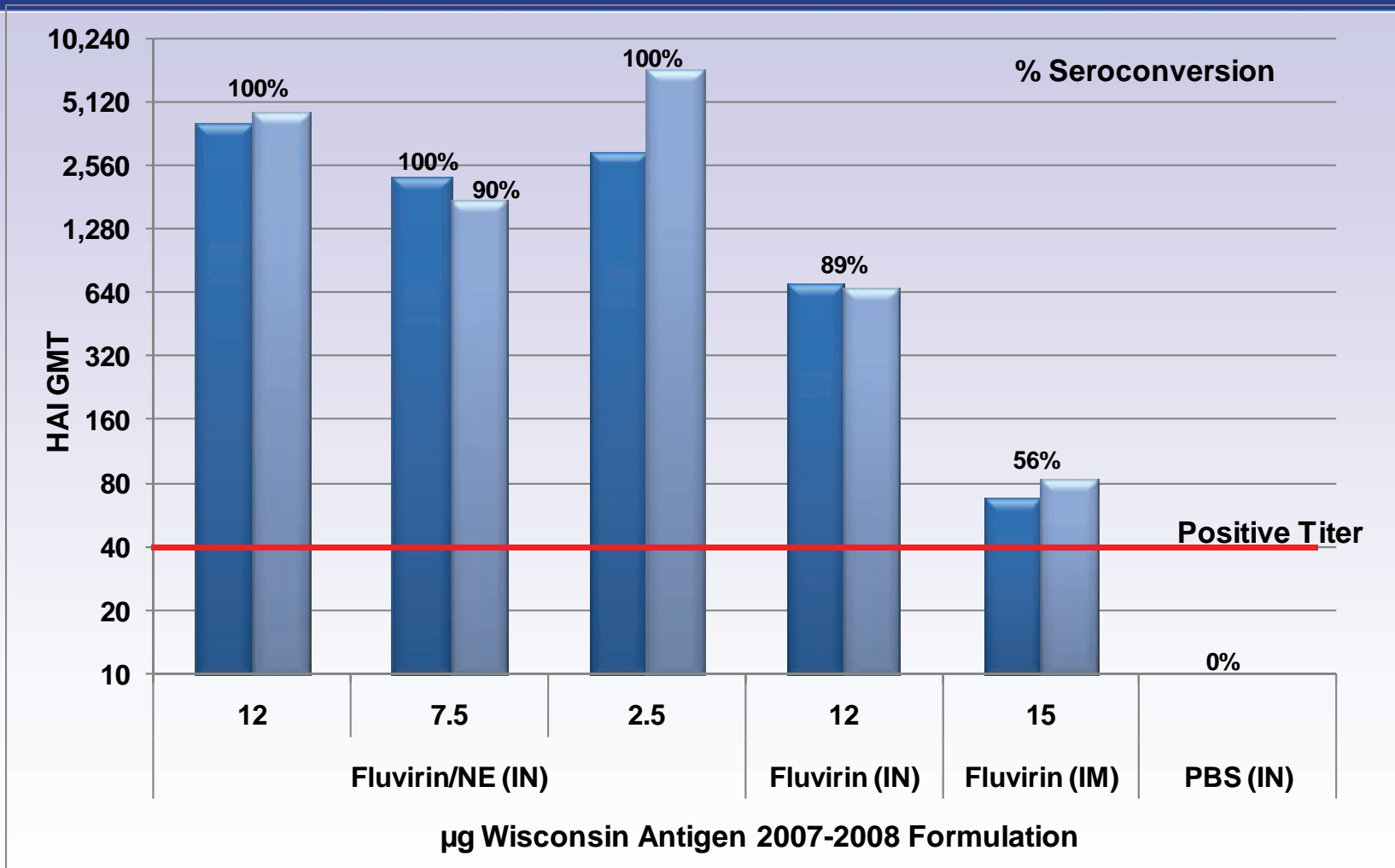


Vaccine	Route	Amount of Influenza (µg HA/ Strain)	Ag Fold-Reduction	Number of Animals
Fluvirin®* + 20% NE**	IN	12	1.25	9
Fluvirin® + 20% NE	IN	7.5	2	10
Fluvirin® + 20% NE	IN	2.5	6	10
Fluvirin®	IN	12	1.25	9
Fluvirin®	IM	9	0	9
PBS	IN	--	--	9

* Fluvirin® 2007-08 formula:
 A/Solomon Islands/3/2006 (H1N1)
 A/Wisconsin/67/2005 (H3N2)
 B/Malaysia/2506/2004

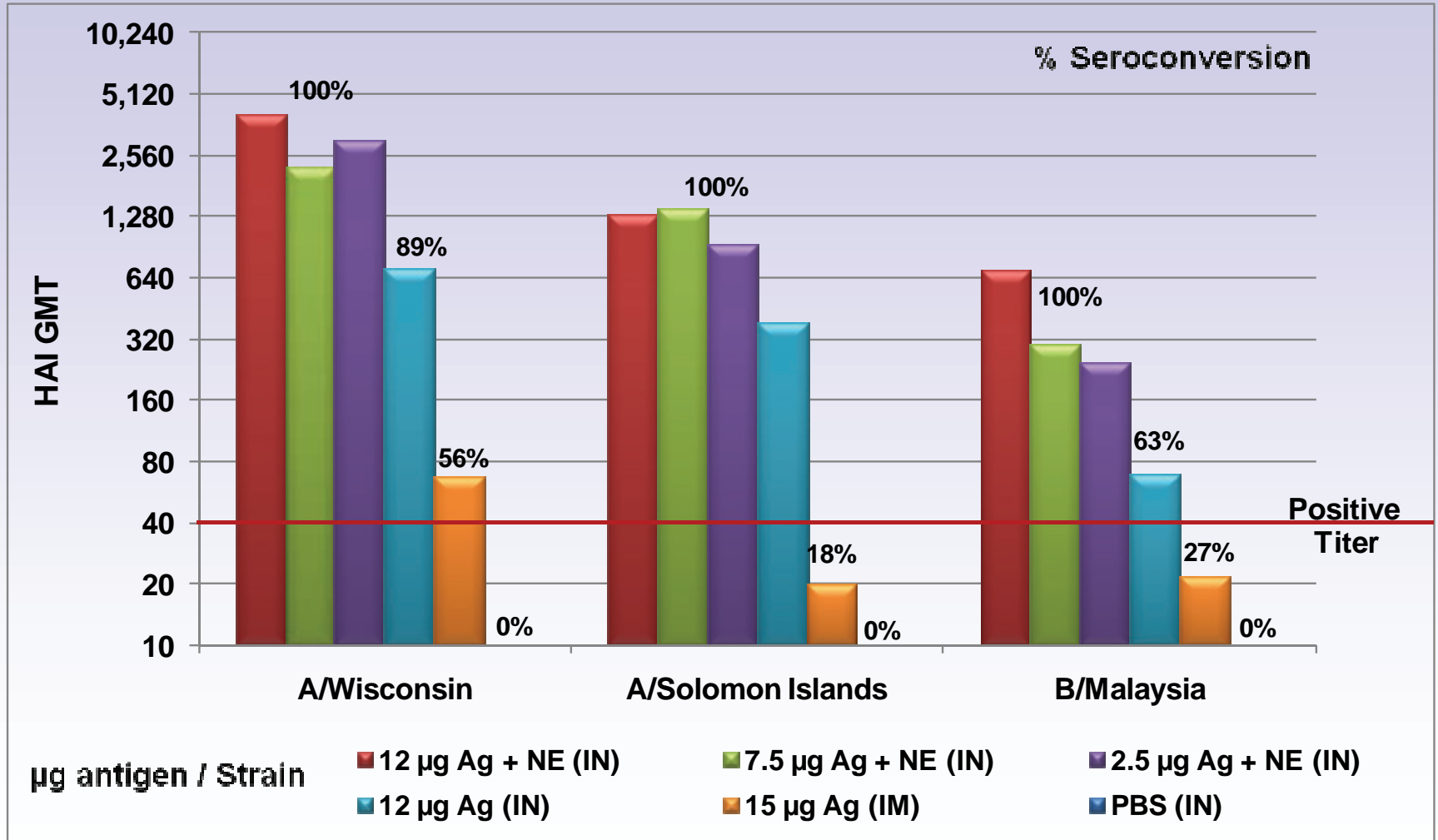
** NE = W₈₀5EC nanoemulsion adjuvant

Ferret HAI GMT to Wisconsin Antigen After One & Two Vaccinations



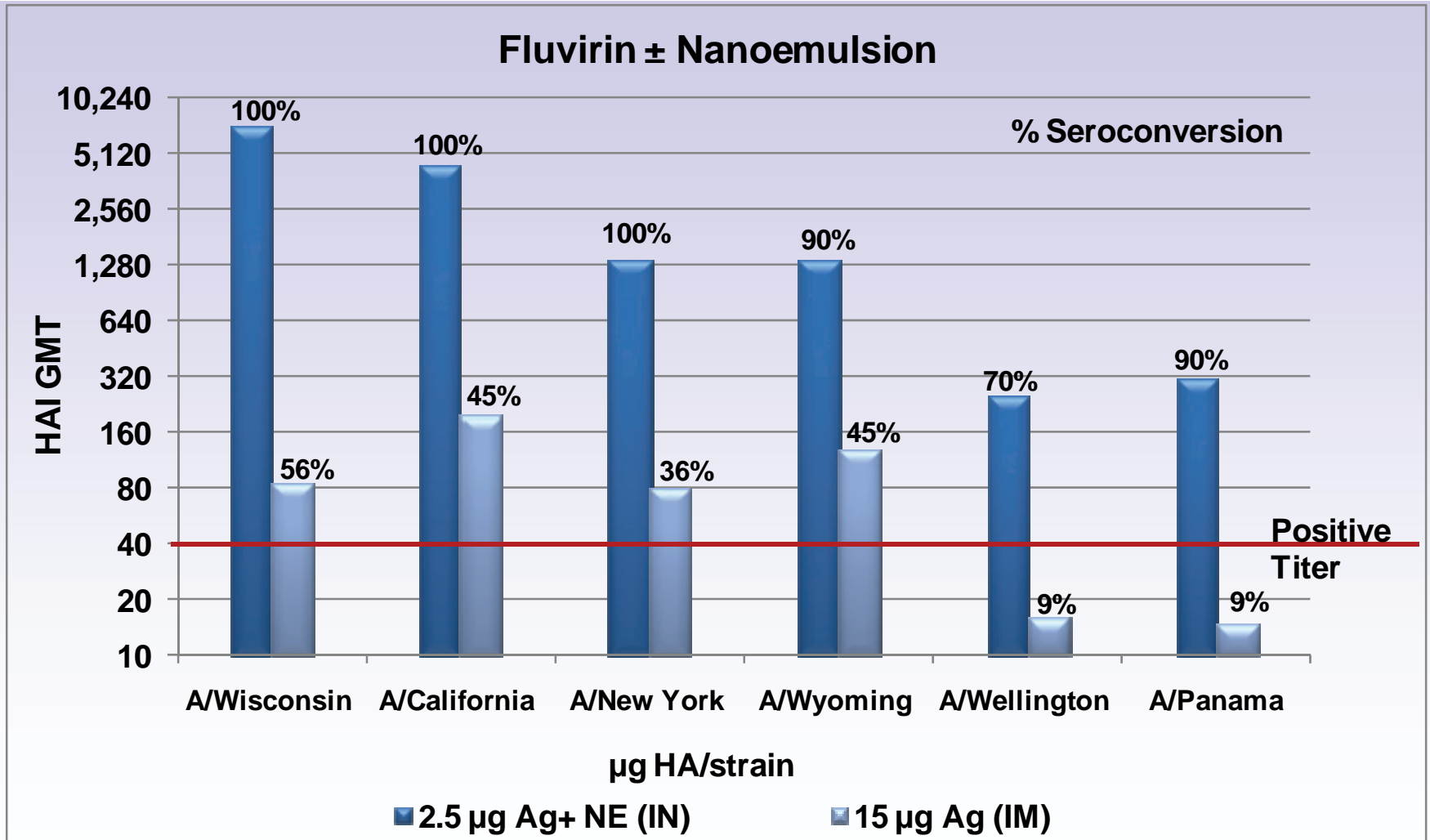
Up to 40x Higher HAI Titers Achieved Using Only 1/6 of the Commercial Antigen Dose Adjuvanted With NE

Ferret HAI GMT to All Three 2007-08 Strains After One Vaccination



High HAI Titers Achieved Against All 3 Strains After a Single Intranasal Vaccination Adjuvanted With NE

Cross Reactivity to H3N2 Antigens Achieved After Two Vaccinations

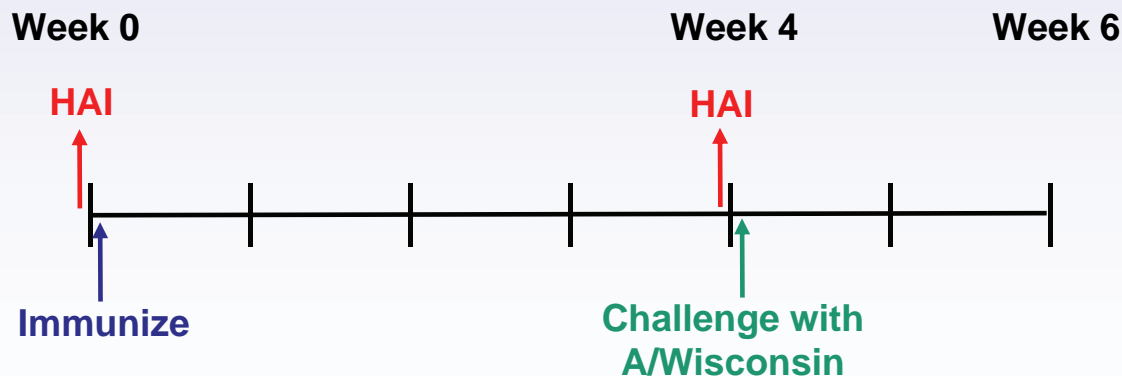


Cross Protection Achieved In 5 Other Influenza Strains Not Present in NE-adjuvanted Vaccine

- **Vaccinated ferrets were challenged with 10^7 EID₅₀ A/Wisconsin virus**
- **No virus detected in nasal washing**
- **No virus detected in nasal turbinates or lung tissues**

- **Purpose:**

- To further demonstrate the dose sparing of the nanoemulsion adjuvant in ferrets (Dose response)
- Demonstrate cross protection and protection against challenge after a single administration of low doses of influenza antigen adjuvanted with nanoemulsion



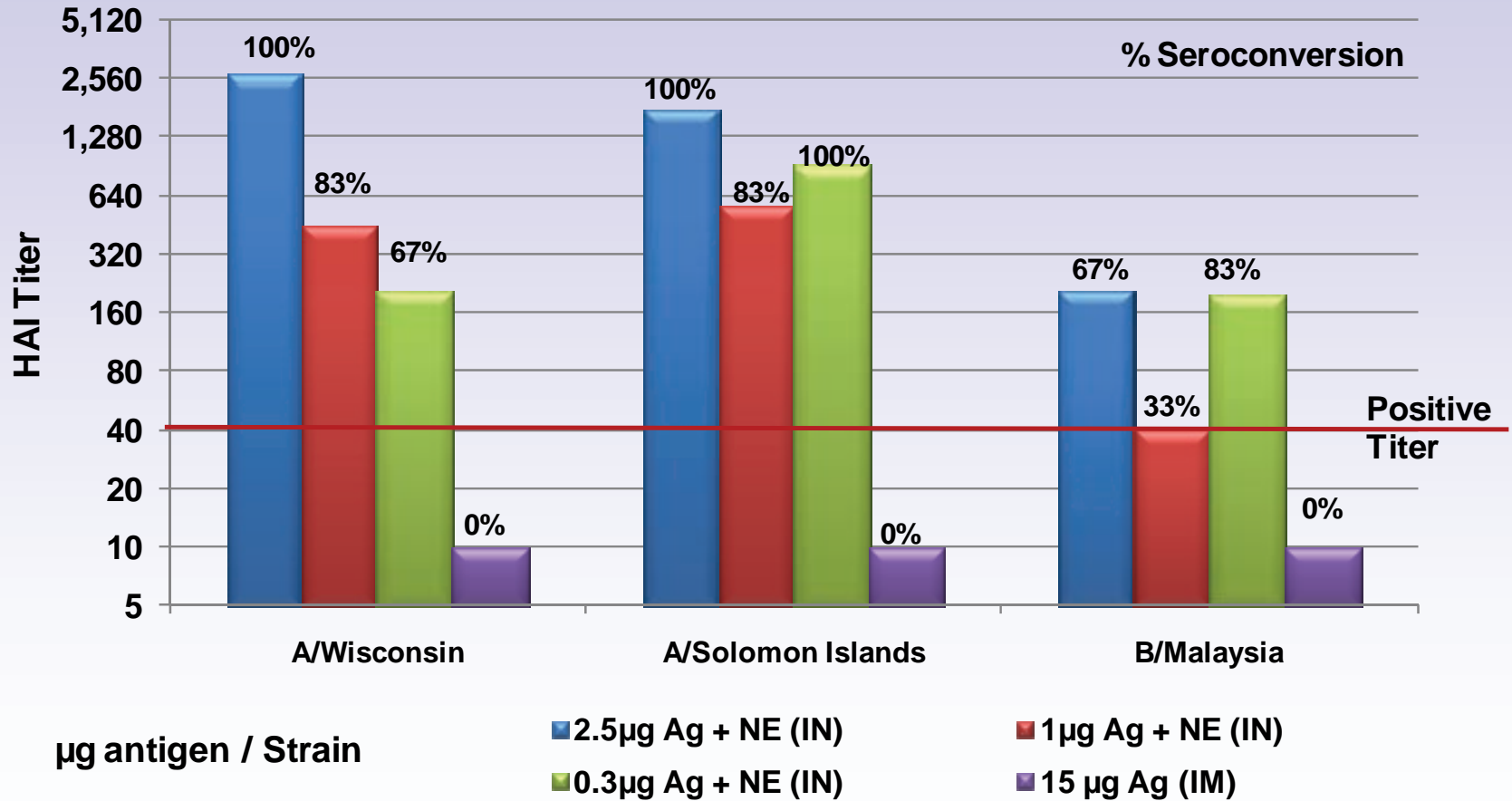
Vaccine	Route	Amount of Influenza (µg HA/ Strain)	Ag Fold-Reduction	Number of Animals
Fluzone®* + 20% NE**	IN	2.5	6	6
Fluzone® + 20% NE	IN	1	15	6
Fluzone® + 20% NE	IN	0.3	50	6
Fluzone®	IM	15	0	6

* Fluzone® 2007-08 formula:
 A/Solomon Islands/3/2006 (H1N1)
 A/Wisconsin/67/2005 (H3N2)
 B/Malaysia/2506/2004

** NE = W₈₀5EC nanoemulsion adjuvant

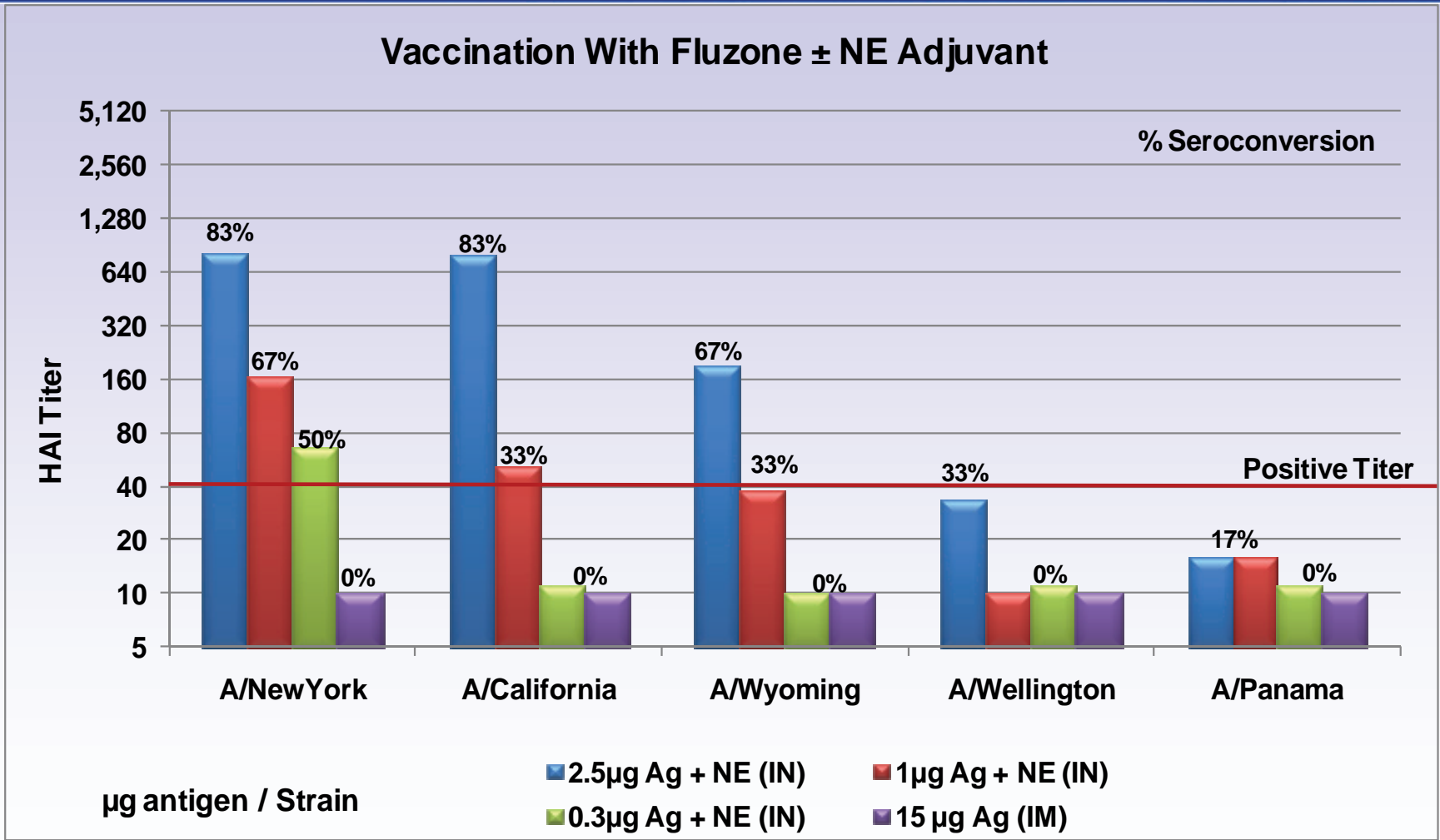
Ferret HAI GMT to All Three 2007-08 Strains After One Vaccination

Vaccination With Fluzone ± NE Adjuvant



High Titers Achieved After a Single Vaccination With 1/50th of the Commercial Antigen Dose Adjuvanted With NE

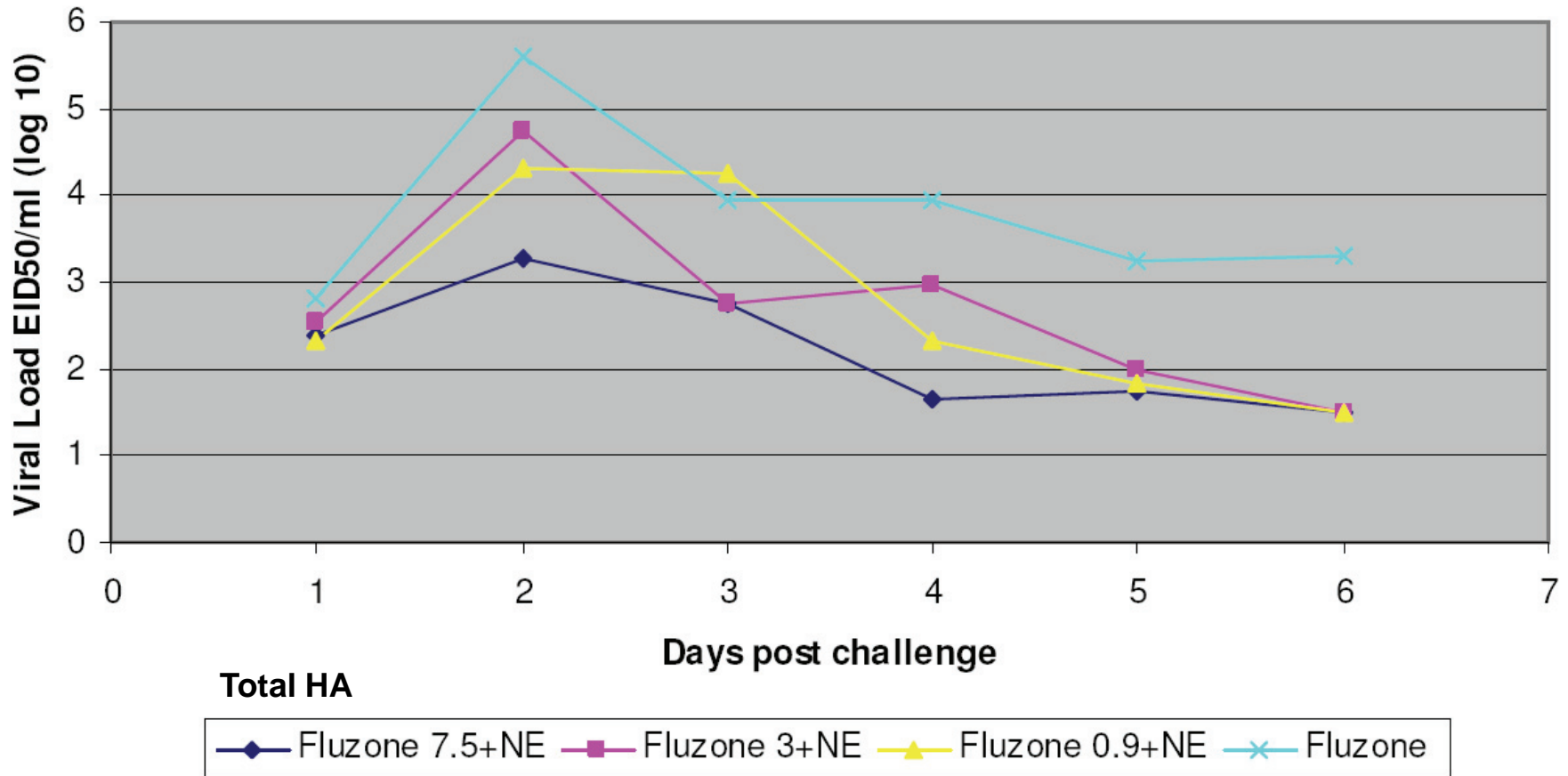
Cross Reactivity to Some H3N2 Antigens Achieved After Only One Vaccination



Cross Protection Achieved In Other Influenza Strains Not Present in NE-adjuvanted Vaccine

Viral Challenge

Viral Load in Nasal Wash of Animals Challenged with 10^7 EID₅₀ of A/Wisconsin Virus



Viral shedding is inversely proportional to antigen dose present in the NE adjuvanted vaccine

Safety Observations From Immunogenicity Studies in Ferrets

- **Four seasonal influenza vaccination studies have been conducted in a total of 354 ferrets:**
 - 249 received NE + antigen intranasally
 - 30 received antigen alone intranasally
 - 54 received IM vaccination without nanoemulsion
 - 21 received PBS
- **NE-adjuvanted vaccines were well tolerated by all animals**
 - No significant treatment-related clinical signs
 - No effects on body weights or temperatures
- **No deaths attributed to NE vaccination**

NE-adjuvanted influenza vaccines:

- Elicit robust immunity after a single dose in naïve ferrets →
Could be effective specifically in children and elderly
- Produce broad cross protection against a range of influenza
A strains
- Markedly reduce the amount of antigen required for effective
vaccination
- Safe to administer nasally

University of Michigan

Paul Makidon

Andrzej Myc

Nick Mank

Jessica Knowlton

Southern Research Institute

Nutan Mytle

Questions

