



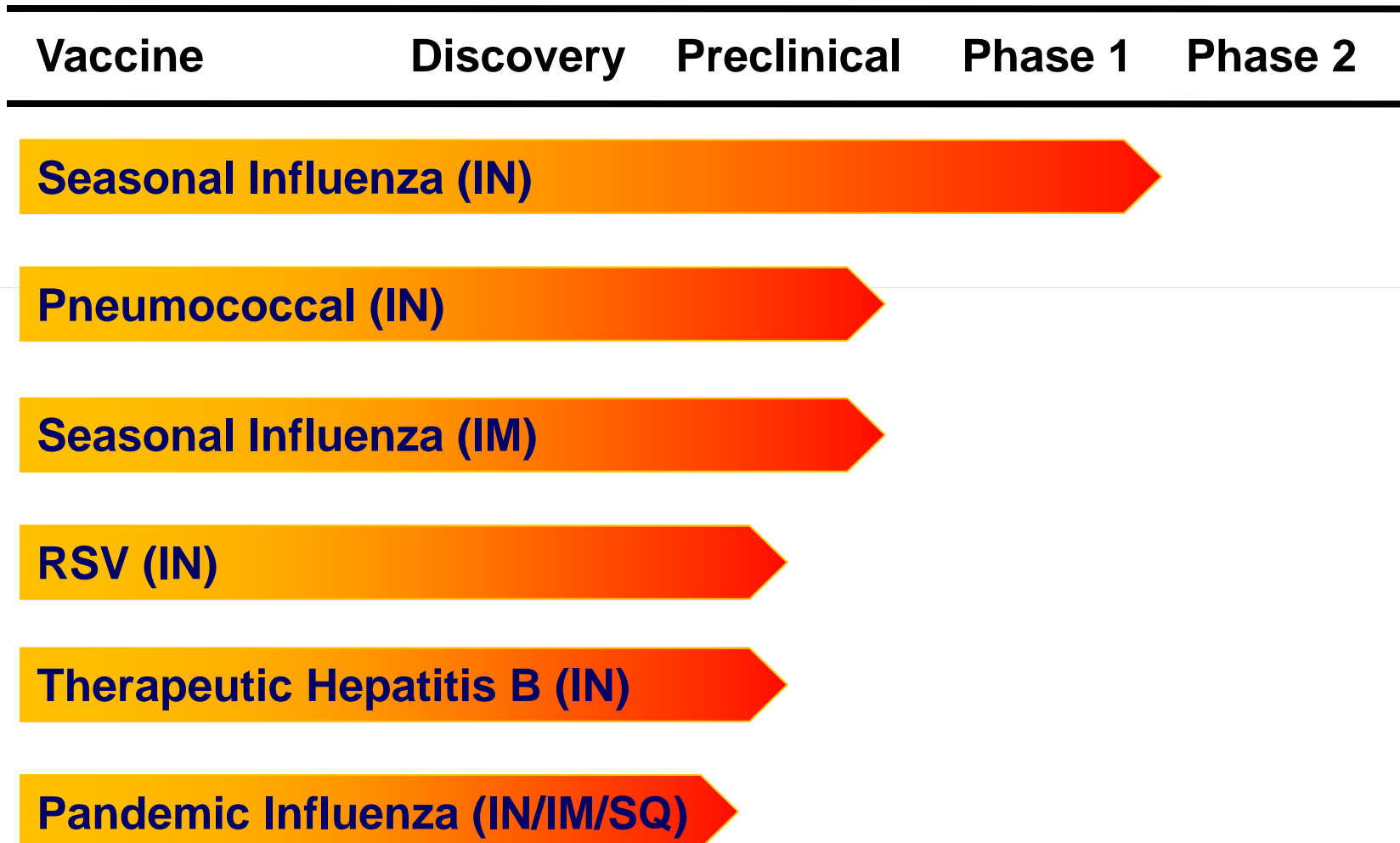
NB-1008 an Intranasal Nanoemulsion Adjuvanted Vaccine – Dose Range Efficacy and Toxicity Studies

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Director of Vaccine Research
NanoBio[®] Corporation

August 19th, 2010

Nanoemulsion (NE) Intranasal Adjuvant Platform

- | | |
|--|--|
| <ul style="list-style-type: none">• High energy emulsification of:<ul style="list-style-type: none">– Water– Oil– Surfactant– Organic Solvent– Cetylpyridinium Chloride
• Average Droplet Sizes<ul style="list-style-type: none">– 400 nm | <ul style="list-style-type: none">▪ <i>Universal</i> adjuvant platform
▪ Advantages:<ul style="list-style-type: none">- 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 |
|--|--|

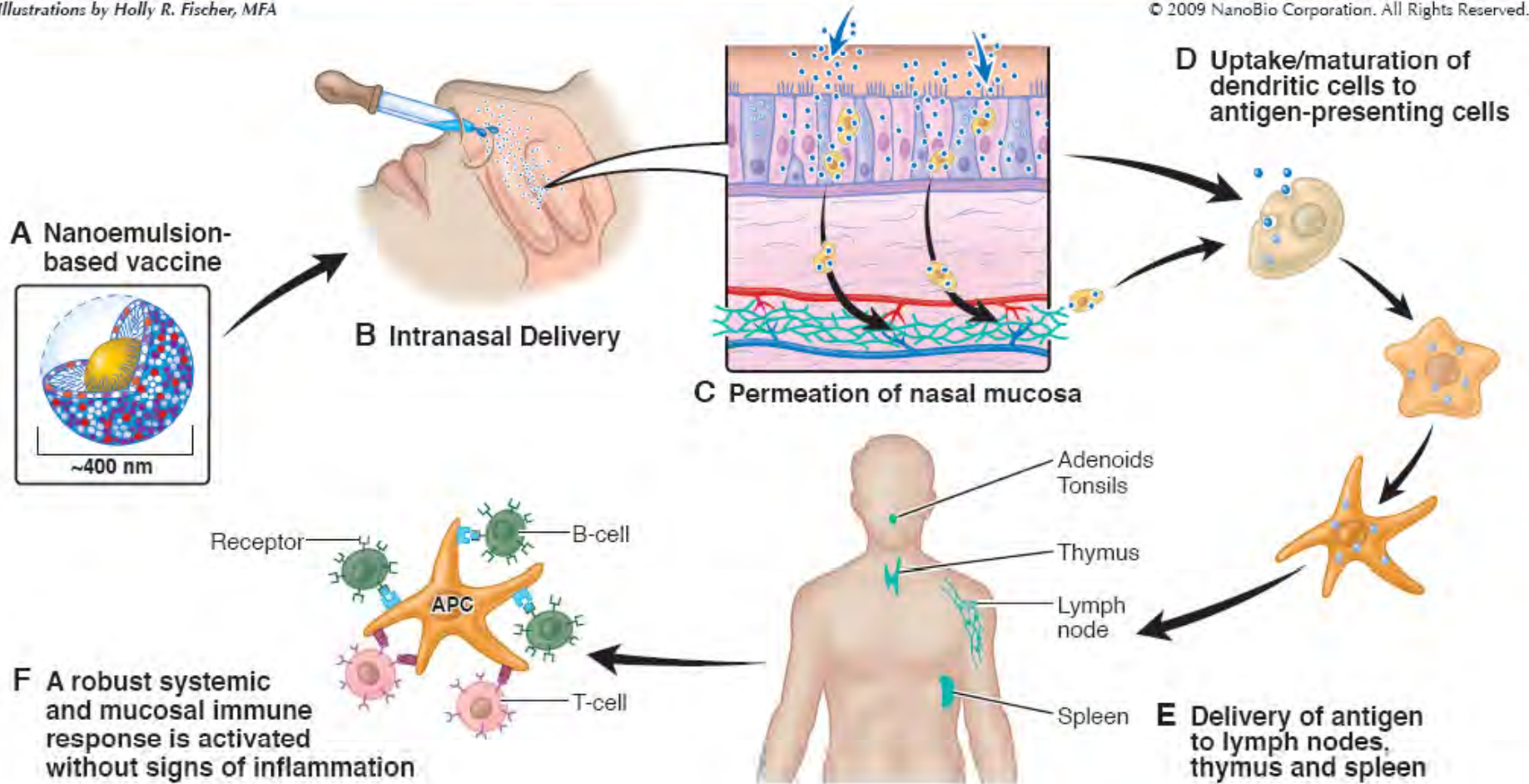


Mechanism of Action

MECHANISM OF ACTION FOR NANOEMULSION-BASED VACCINES

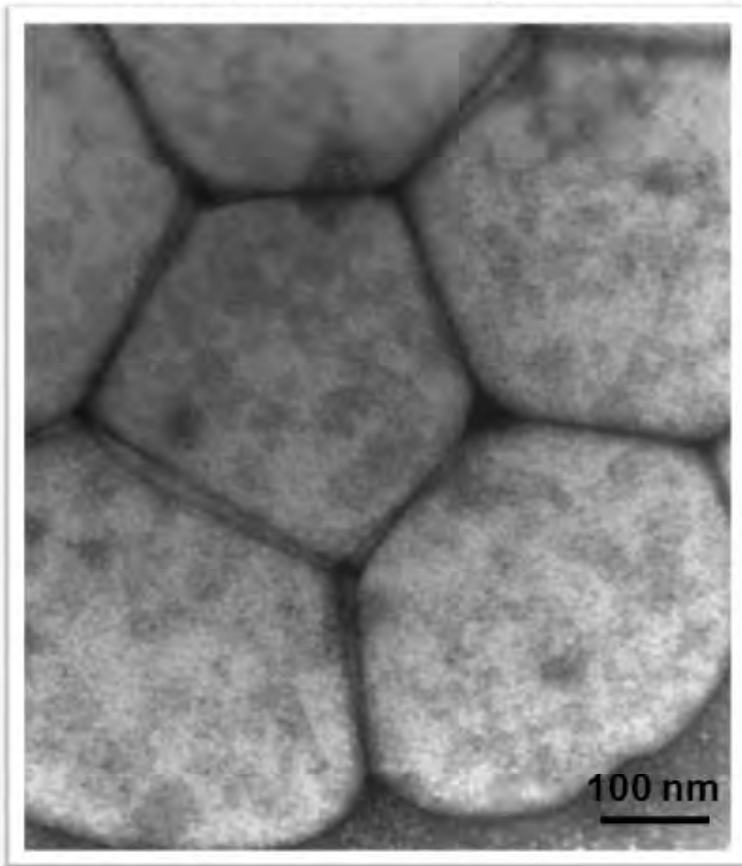
Illustrations by Holly R. Fischer, MFA

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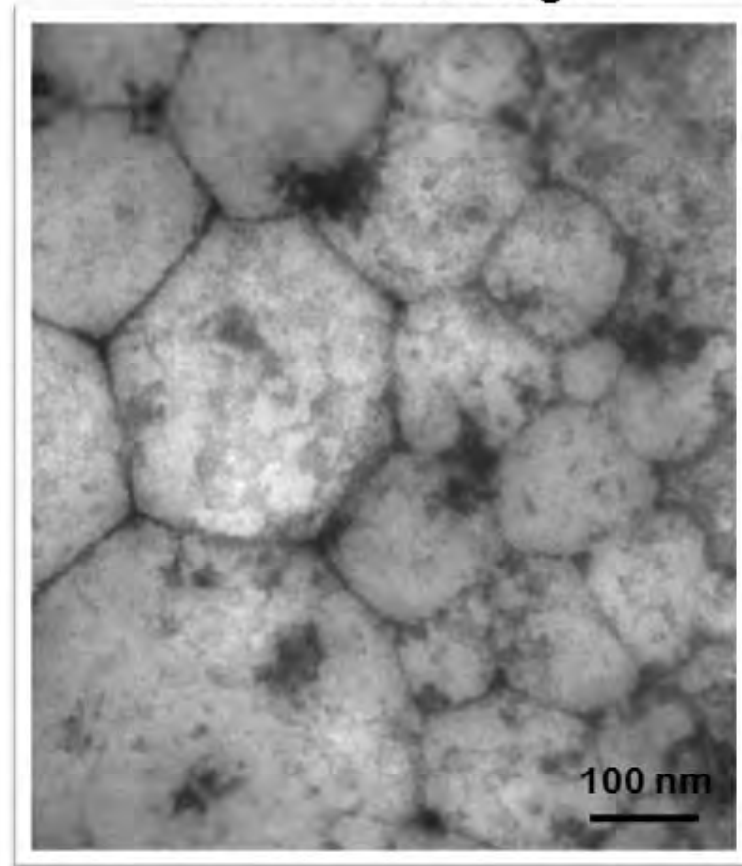


NE Adjuvanted Influenza Antigen

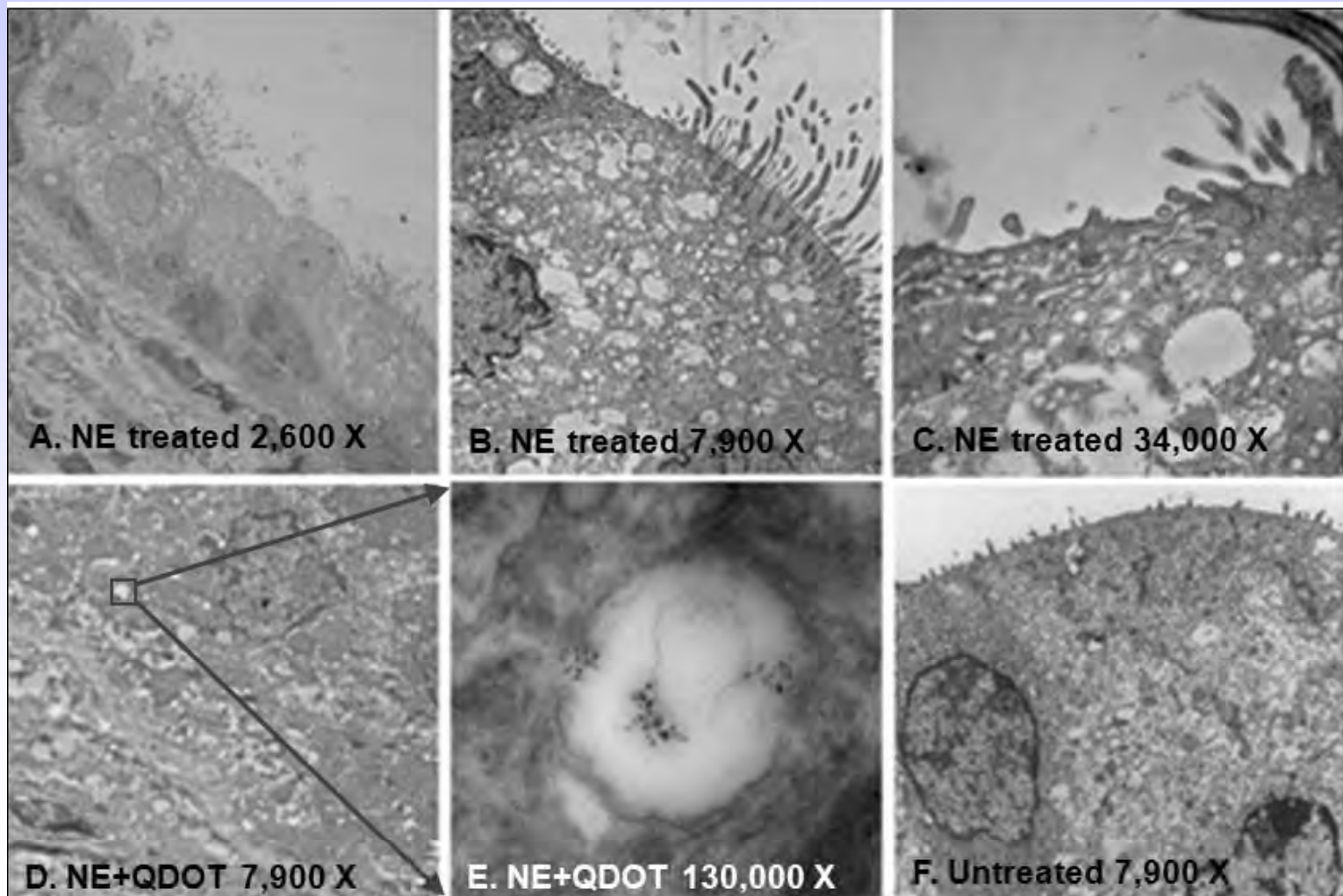
Nanoemulsion Adjuvant



**Nanoemulsion Adjuvant Mixed
With Influenza Antigen**



NanoBio Corporation Ultrastructure of the Nasal Epithelium



Studies performed at the University of Michigan, Nanotechnology Institute for Medicine and Biological Sciences

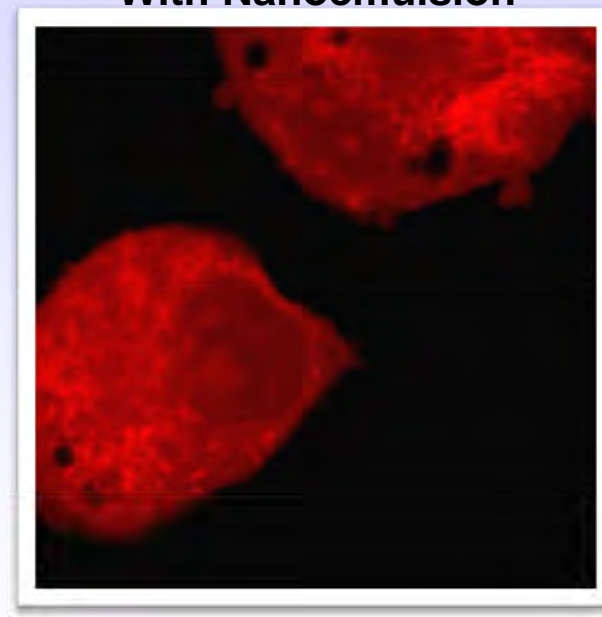
Nanoemulsion Specifically Taken Up By Dendritic Cells

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

Without Nanoemulsion



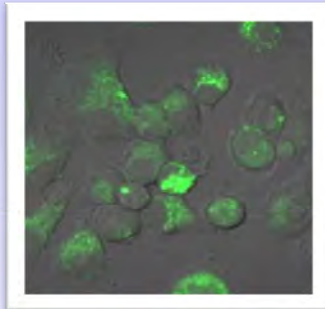
With Nanoemulsion



Nanoemulsion Enhances Antigen Internalization into Dendritic Cells

Recombinant Protective Antigen of Anthrax (rPA)

rPA-FITC / NE



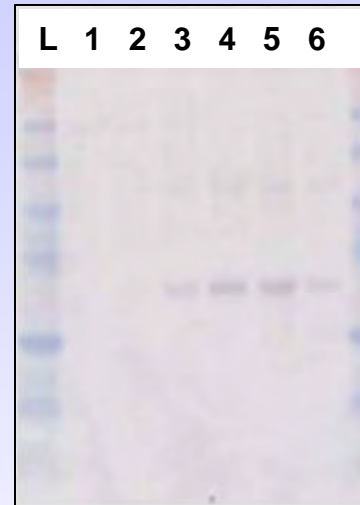
rPA-FITC



Control



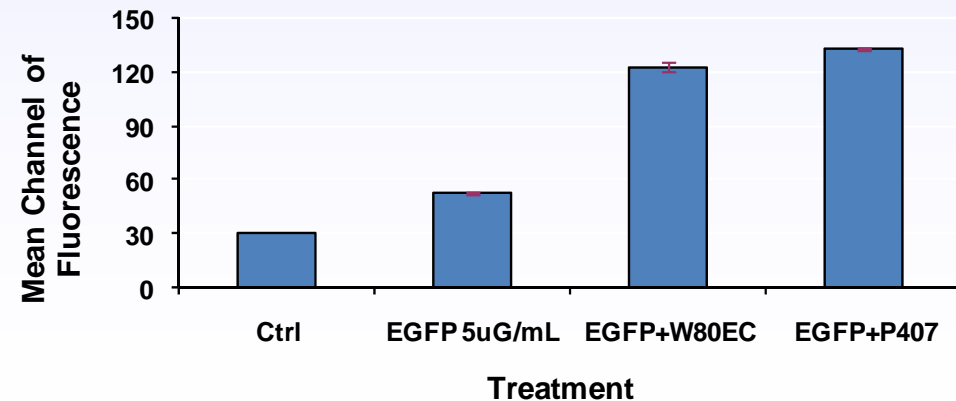
HBsAg Uptake – Western Blot



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

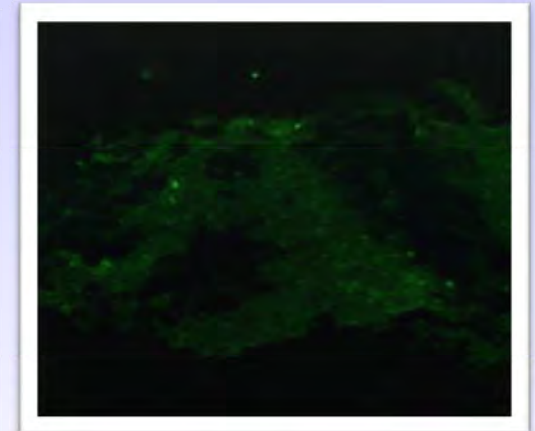
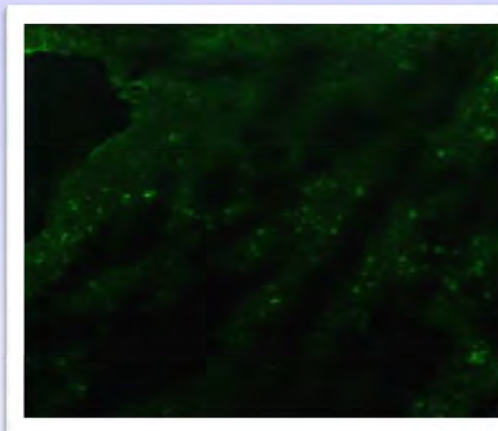
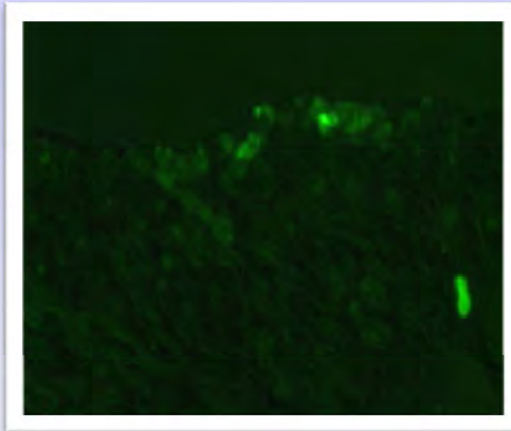
After 24 Hours

Nasal Epithelium

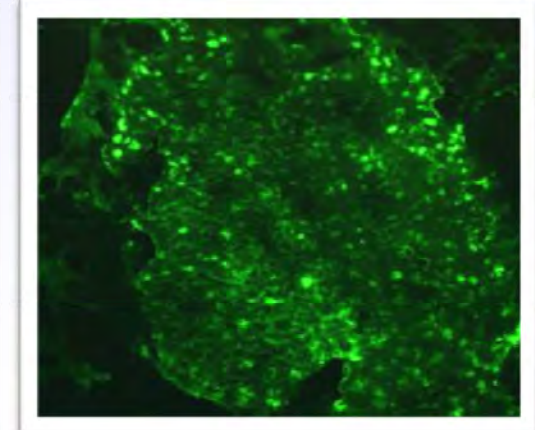
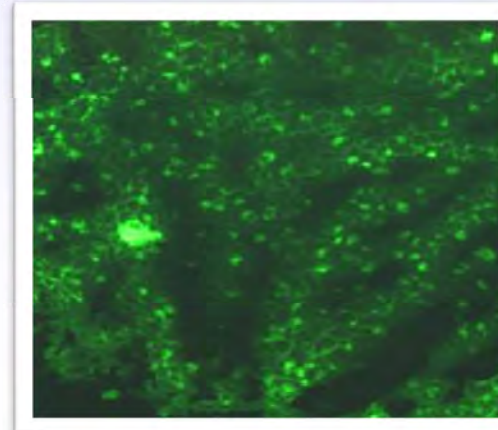
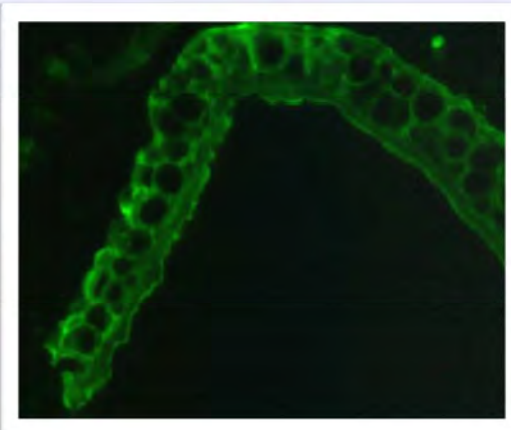
Submandibular Lymph Nodes

Thoracic Lymph Node

GFP Alone



GFP With NE



Nanoemulsion Enhances Delivery to Immune System

QDOT Without Nanoemulsion

QDOT With Nanoemulsion

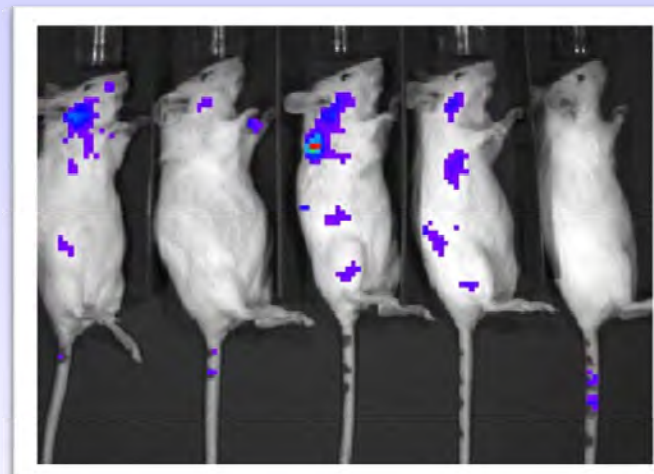
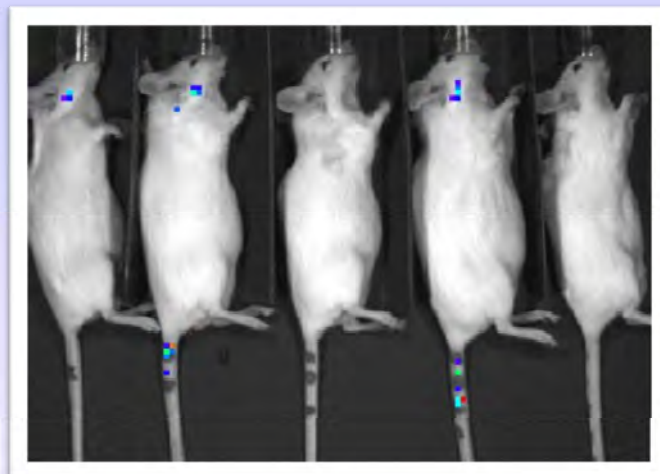
QDOT + PBS

Naive

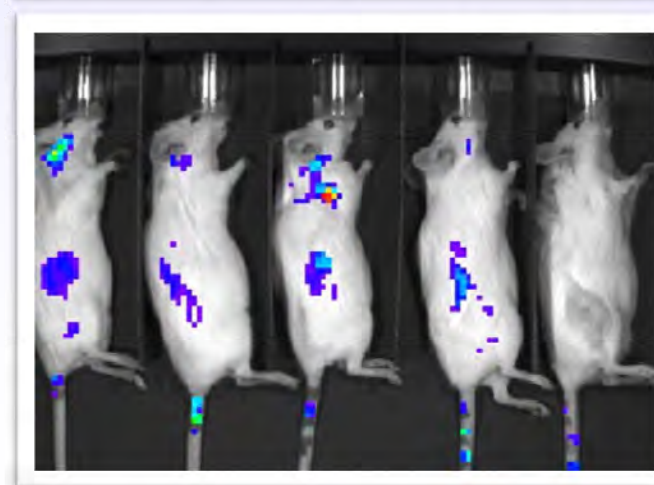
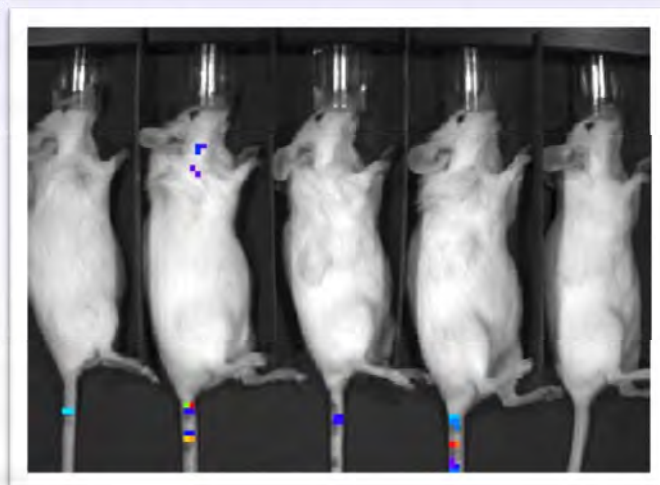
QDOT + NE

Naive

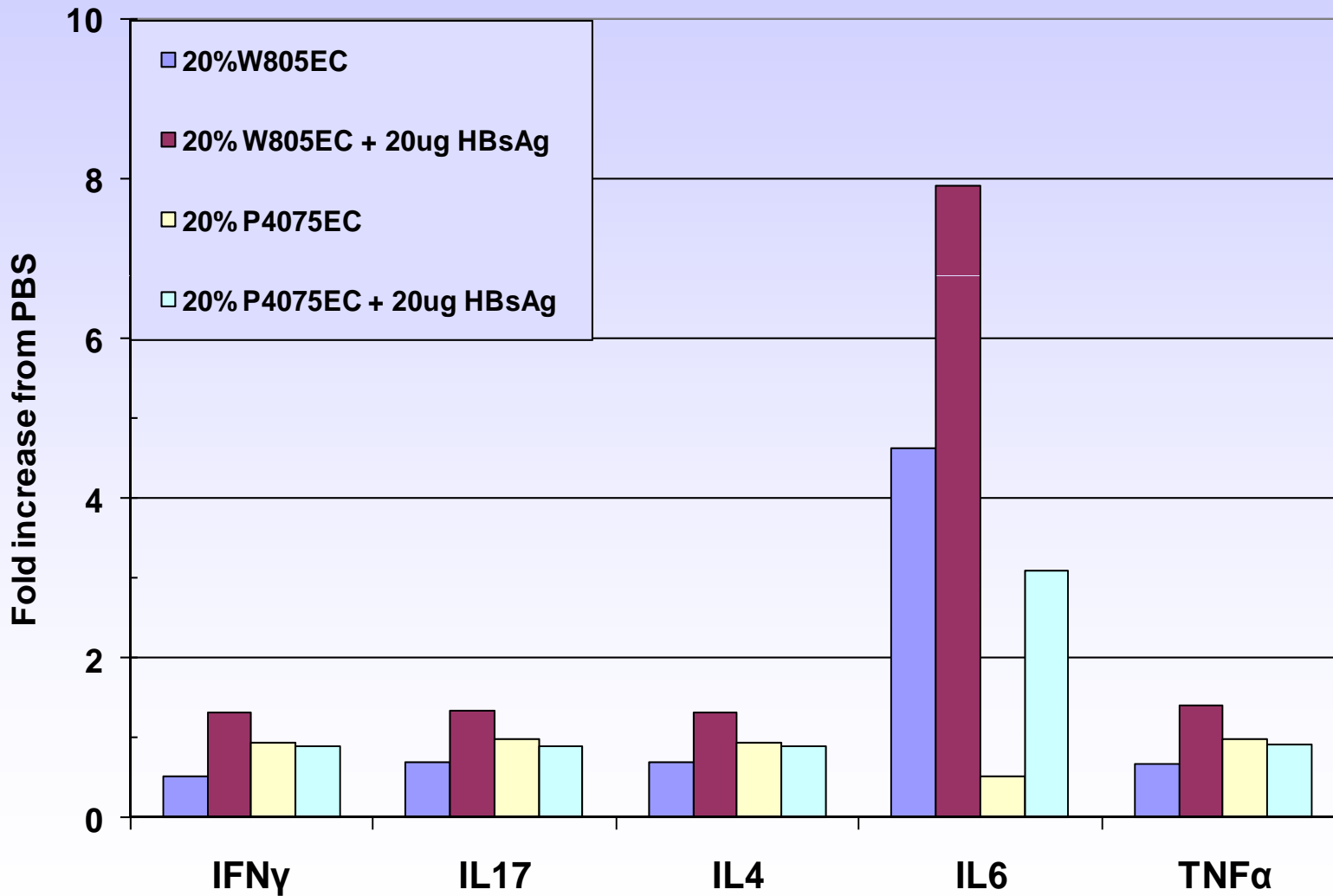
24 Hours



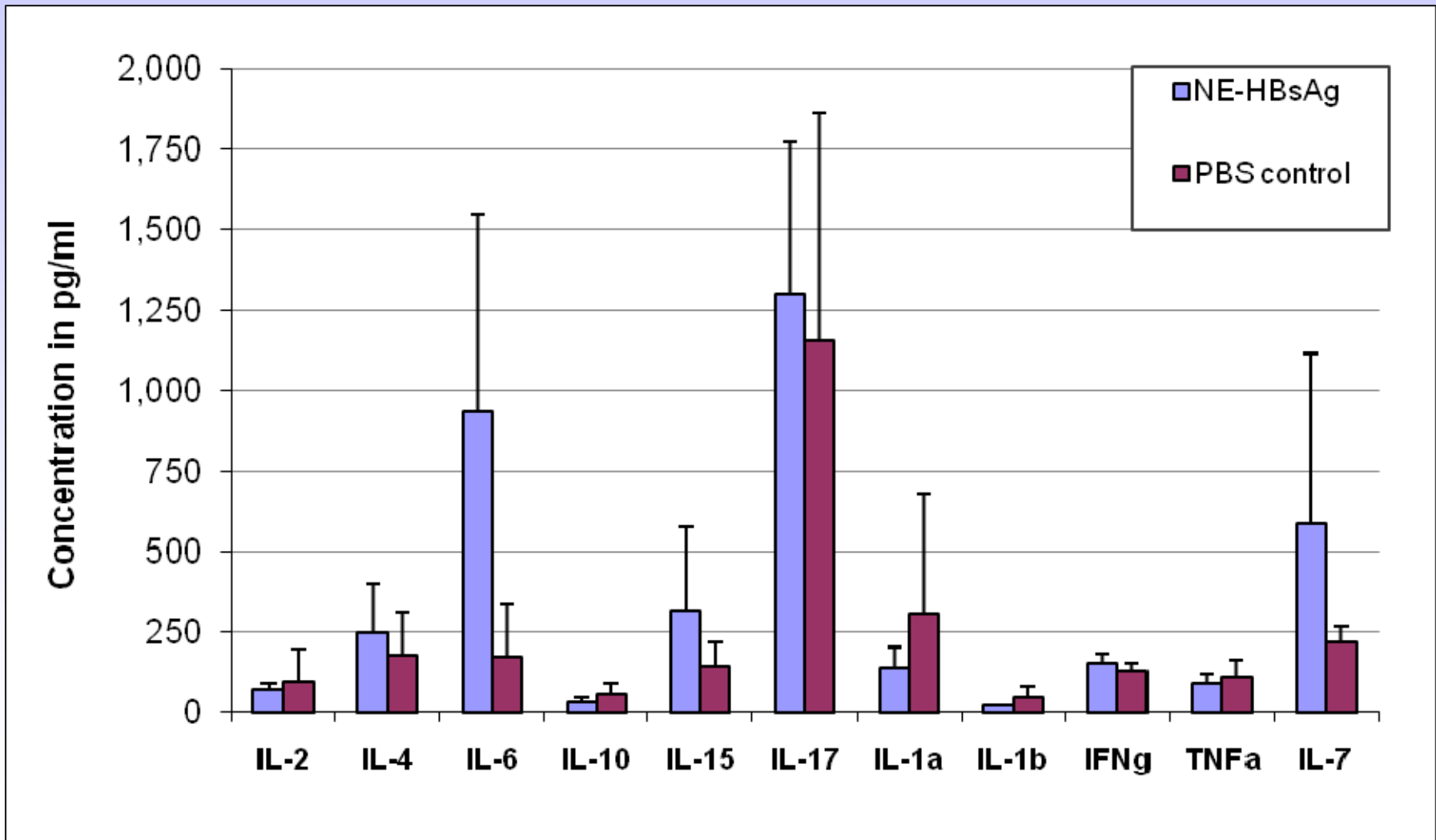
72 Hours



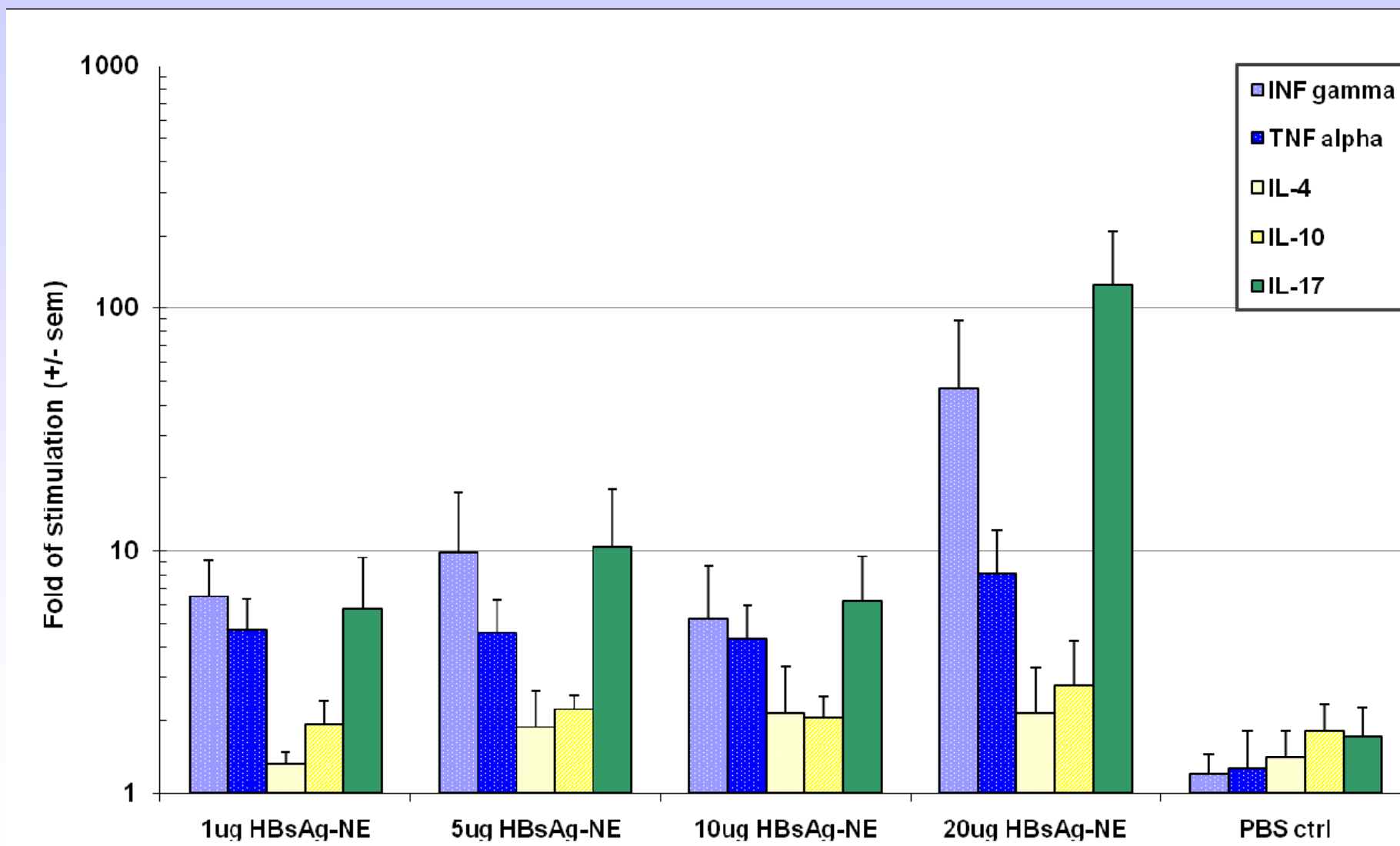
Cytokines Present in BAL 6 Hours Following Vaccine Administration



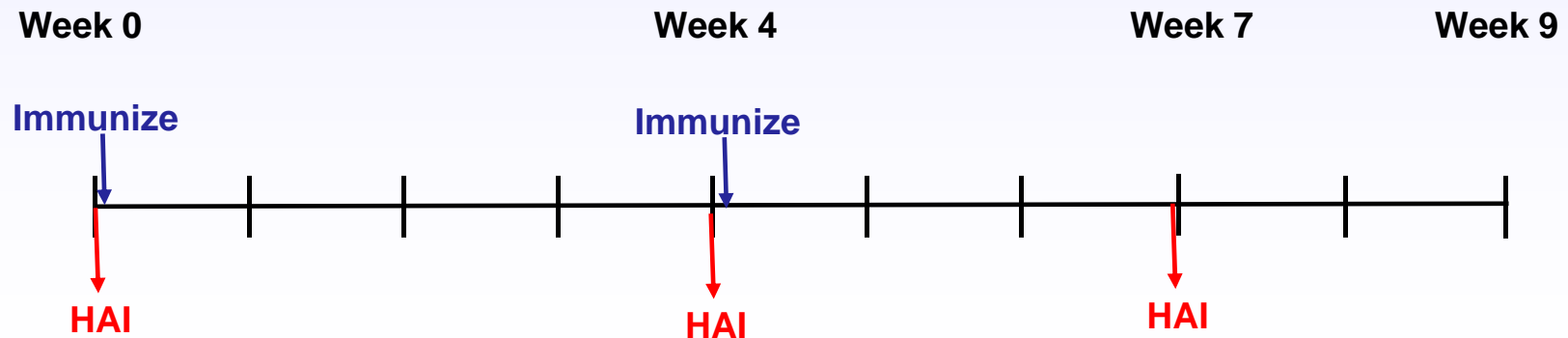
Cytokines in Serum 24 Hours Following NE Nasal Vaccine



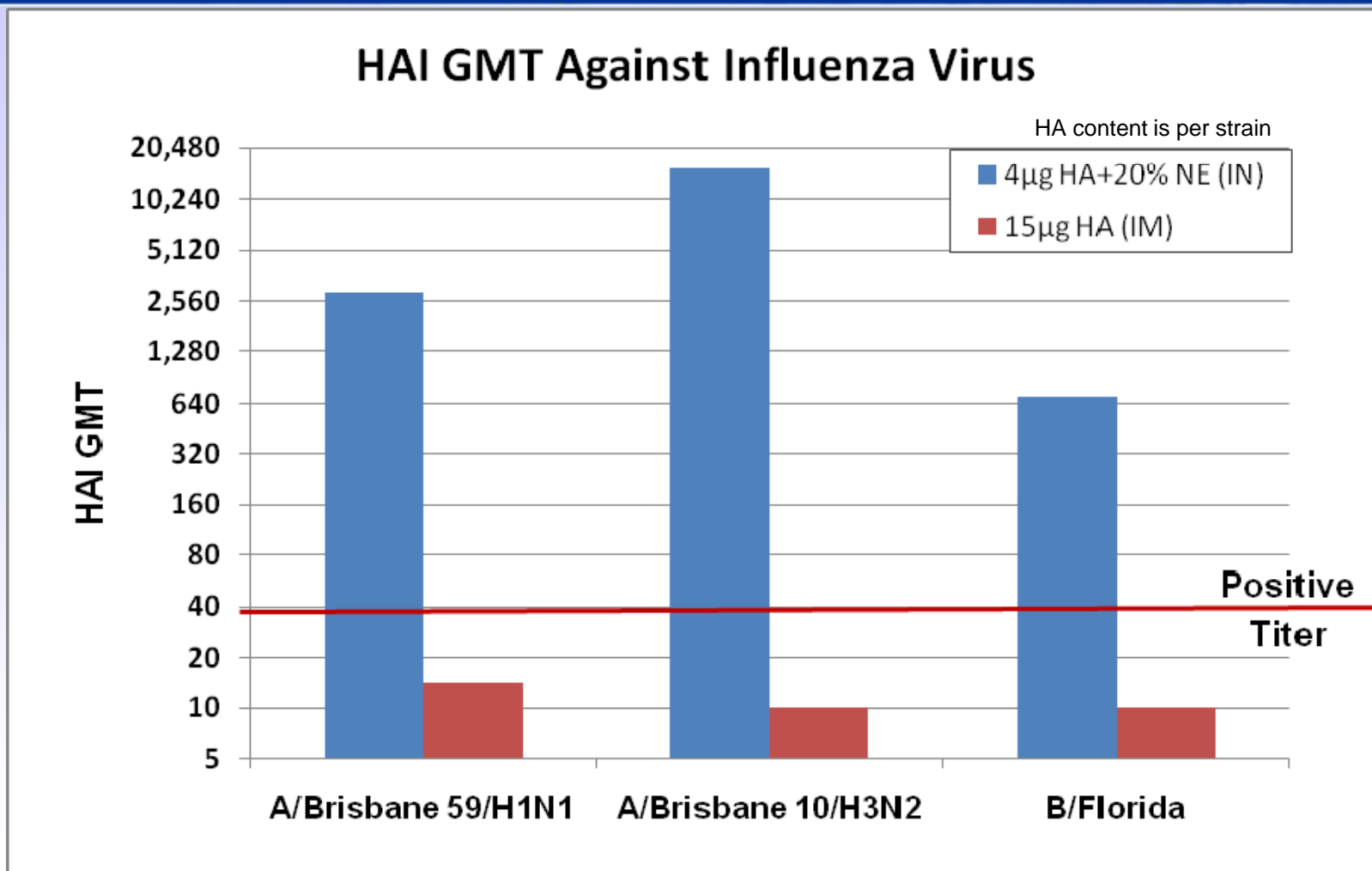
Antigen-specific TH1, TH2 and TH17 Type Cytokine Expression in Splenocytes



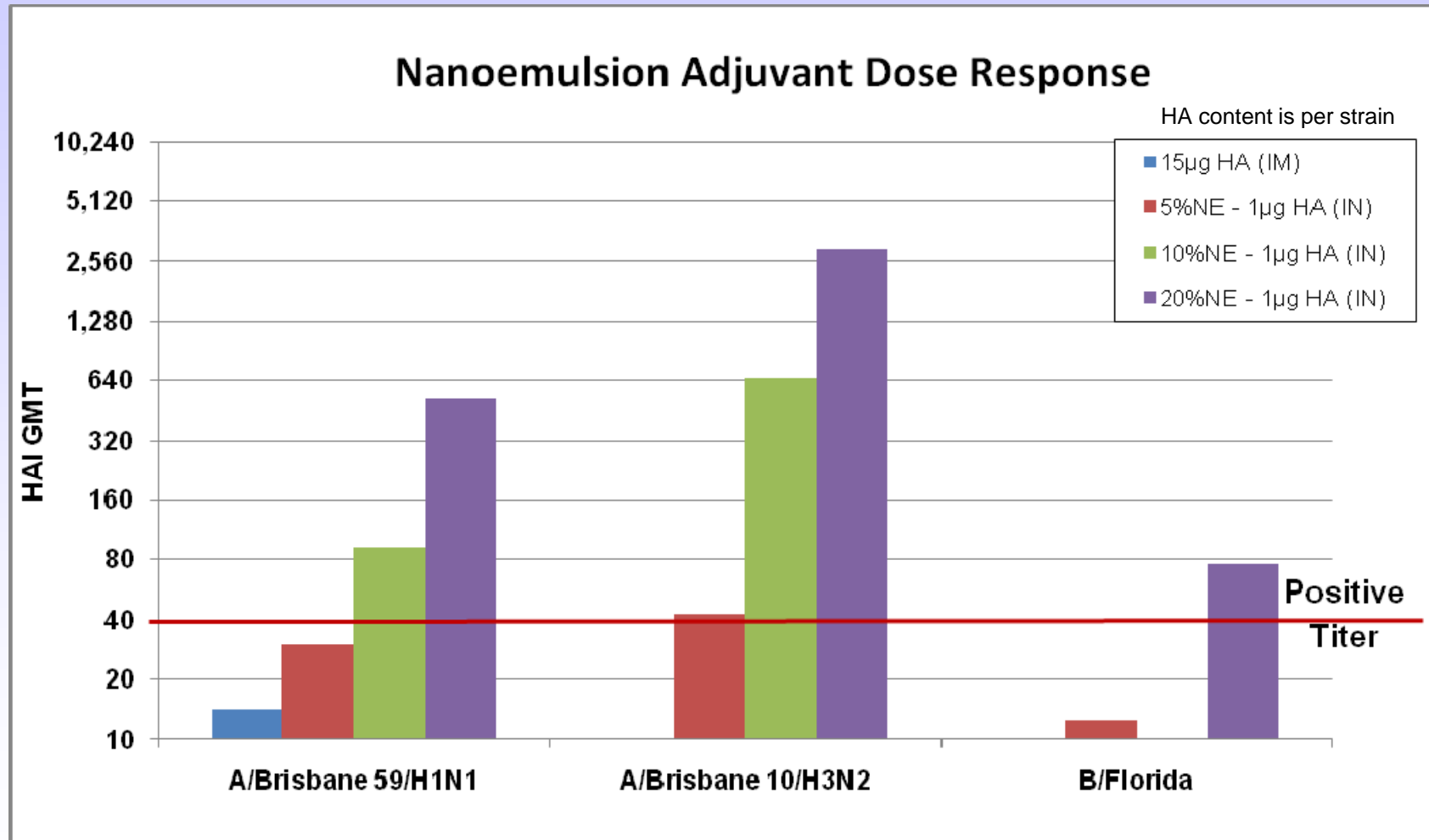
- **Purpose:**
 - Demonstrate immune response with influenza commercial vaccine (Fluzone®) adjuvanted with nanoemulsion (NE)
 - Assess intranasal administration compared to IM
 - Demonstrate the dose sparing effect of the NE adjuvant
 - Different antigen dose (1-4 µg)
 - Different vaccine volume (100 – 500 µL)
 - Different adjuvant concentration (5 – 20%)



High Immune Response Following A Single Dose of Adjuvanted Fluzone® Intranasally

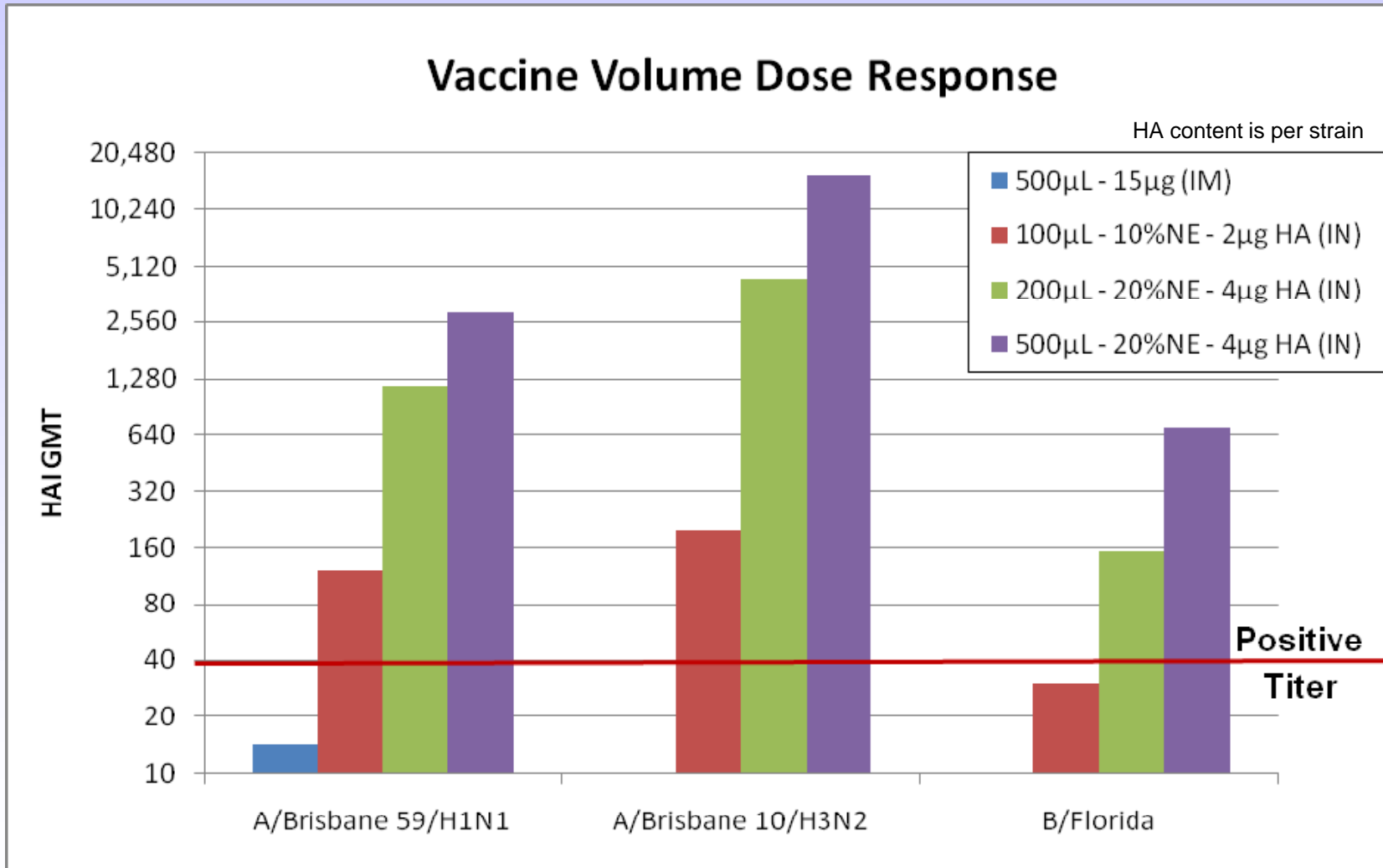


3.75 Fold Dose Sparing Effect of the Nanoemulsion
With Thousand Fold Increase in Immune Response



HAI GMT is Dependent on Nanoemulsion Adjuvant Concentration

Vaccine Volume Dose Response



HAI GMT is Dependent on Vaccine Volume and Antigen Concentration

Preclinical Safety of Nanoemulsion After Nasal Administration

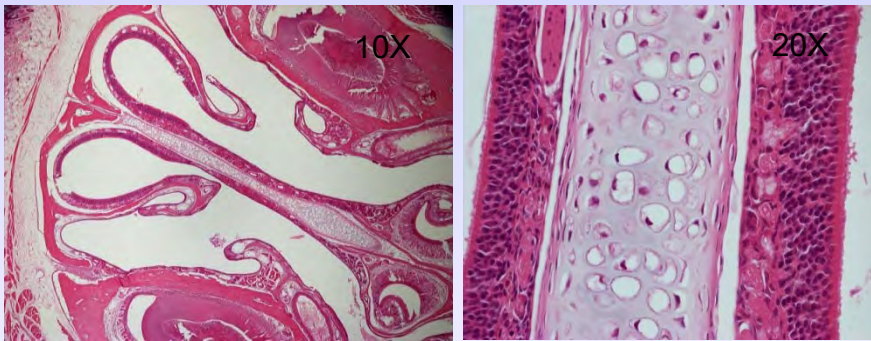
- **Species evaluated:**
 - mice, rats, guinea pigs, ferrets, dogs and primates
- **Morbidity:**
 - no changes in body weight and temperature after multiple intranasal administrations
- **Normal histopathology of nasal cavities and lungs**
 - Complete absence of inflammation
- **Inflammatory cytokine production in nasal wash and bronchial lavage**
 - Showing only induction of IL-6
- **No metabolic changes observed**

Normal Histology of Nasal Epithelium After Vaccine Administration

24 hours

14 days

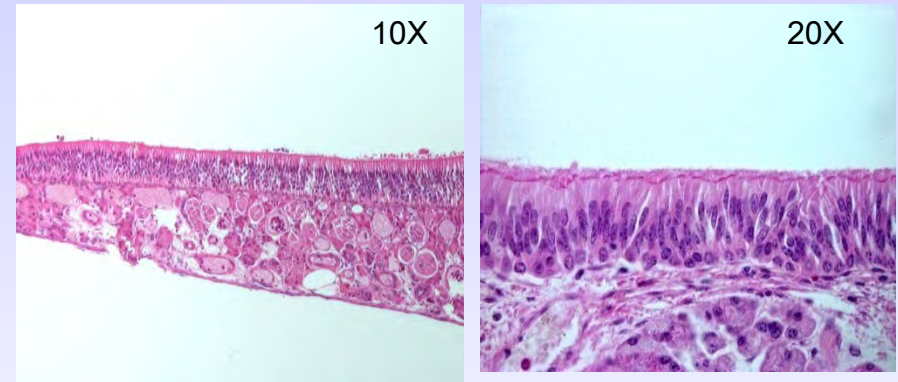
Mouse



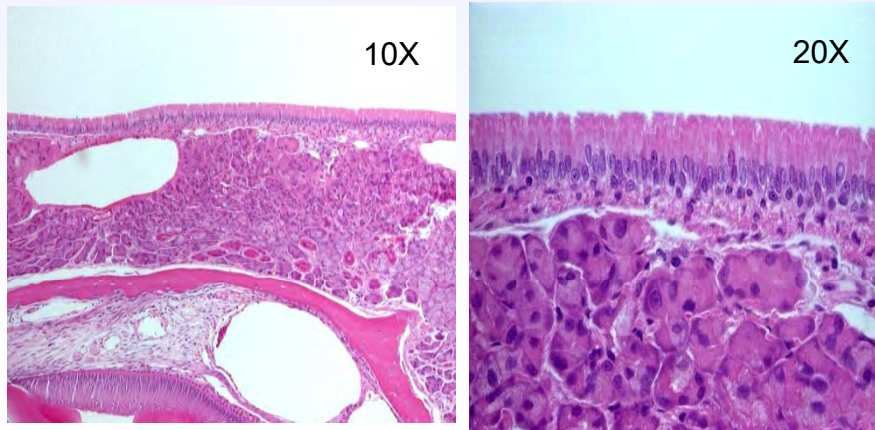
24 hours

14 days

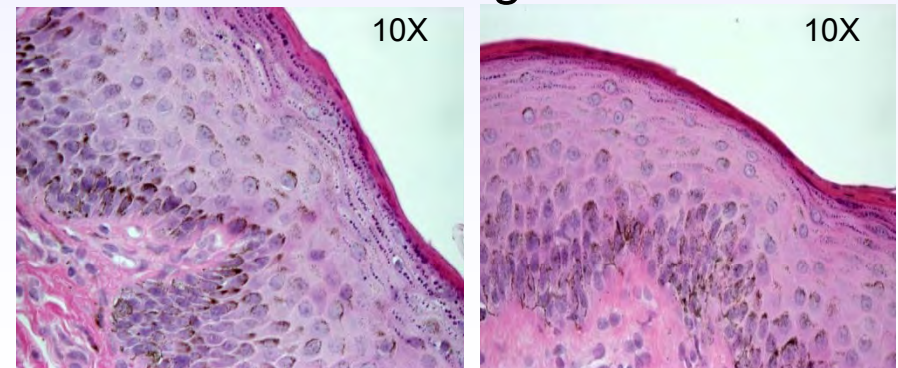
Rat



Guinea pig



Dog



Rabbit GLP Study for NE-Adjuvanted Influenza Vaccine

Treatment	Amount of Influenza Antigen (Total µg HA) ^a	Route	N (F/M)	# of Doses ^b	Total Volume (250µL/nare)
PBS	0	IN	5/5	2	500
Commercial Vaccine + 10% NE	15	IN	10/10	2	500
Commercial Vaccine + 10% NE	30	IN	10/10	2	500
10% NE	0	IN	5/5	2	500
Commercial Vaccine + 20% NE	15	IN	10/10	2	500
Commercial Vaccine + 20% NE	30	IN	10/10	2	500
20% NE	0	IN	5/5	2	500
Commercial Vaccine	30	IN	10/10	2	500

a A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2) and B/Florida/04/2006.

b Doses were administered on Day 0 and 14. Samples for HAI titers were collected on Day 1, 16 and on Day 29.

- **Clinical Observations**
- **Ophthalmoscopy**
- **Body weights and food consumption**
- **Body temperatures**
- **Serum chemistry**
- **Hematology**
- **Coagulation and urinalysis**
- **Organ weights and organ weight ratios**
- **Gross and microscopic pathology**
- **Examination of sections of nasal turbinates, cribriform plate, olfactory bulb, brain, pituitary and cranial nerves**

- **No changes:**

- clinical signs,
- food consumption,
- ophthalmology.
- body weight,
- temperature,

- **Changes not considered adverse**

- absolute neutrophil count
- fibrinogen

- **Minimal to mild inflammation**

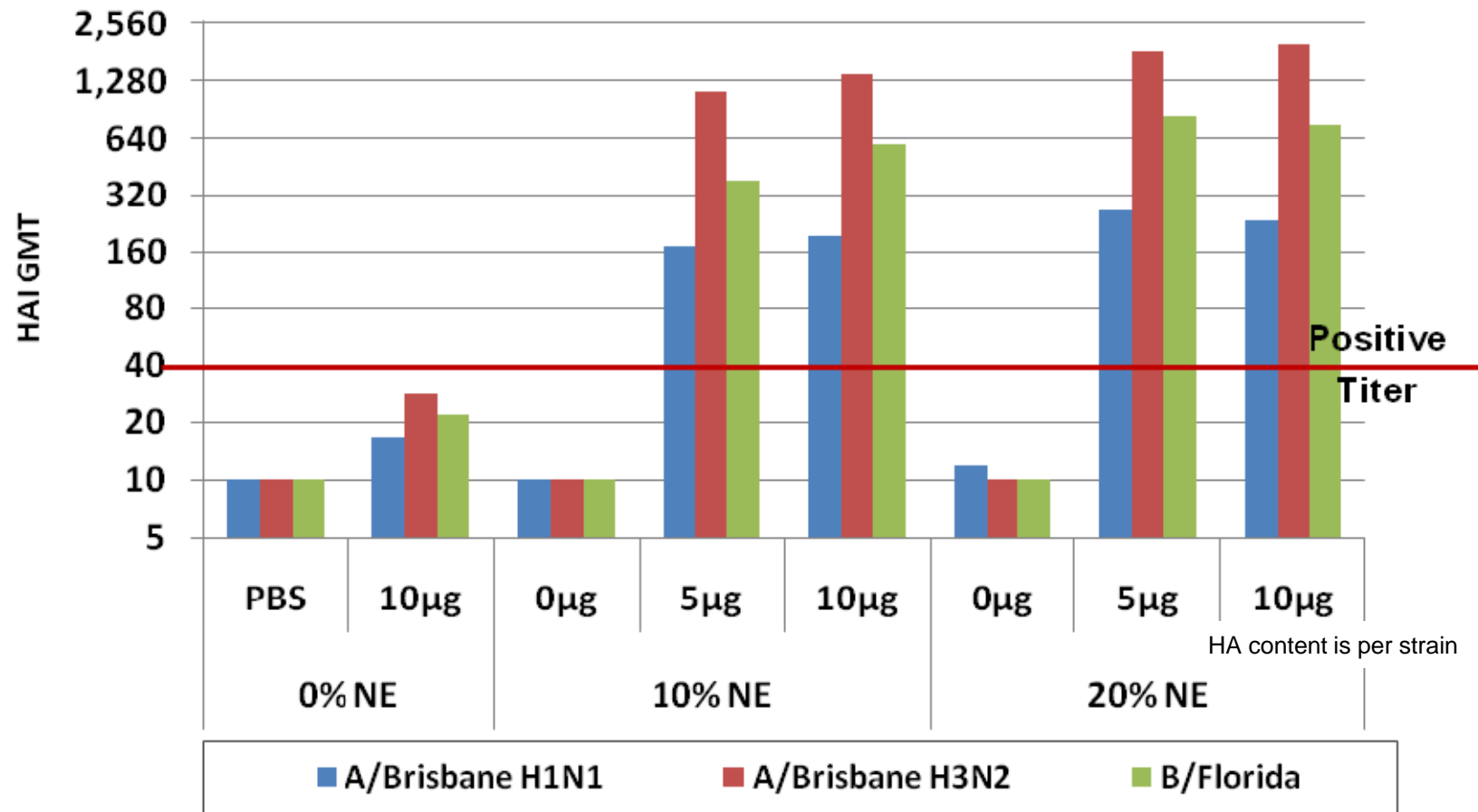
- nasal turbinates

- **No inflammation observed in**

- brain,
- cribiform plate,
- trigeminal or facial nerves.
- pituitary,
- olfactory bulbs,

Rabbits Immune Response Following NE-Adjuvanted Fluzone®

Rabbit Immune Response On SD29



Rabbits Responded to Nanoemulsion Adjuvanted Fluzone®

- **NB-1008 was administered intranasally to 140 subjects**
- **No serious AEs, no AEs leading to study discontinuation**
- **AE's were generally mild to moderate and equally distributed between intranasal groups**
 - **20% of subjects receiving intranasal administration had transient nasopharyngeal irritation**
 - **60% of subjects receiving intramuscular administration had pain at the injection site**
- **Efficacy data will be presented at ICAAC next month.**

NE-adjuvanted influenza vaccines:

- Elicit robust Th1 immune response
- 15-Fold dose sparing effect for commercial vaccine
- Safe to administer nasally
- Phase I clinical study is ongoing

Questions