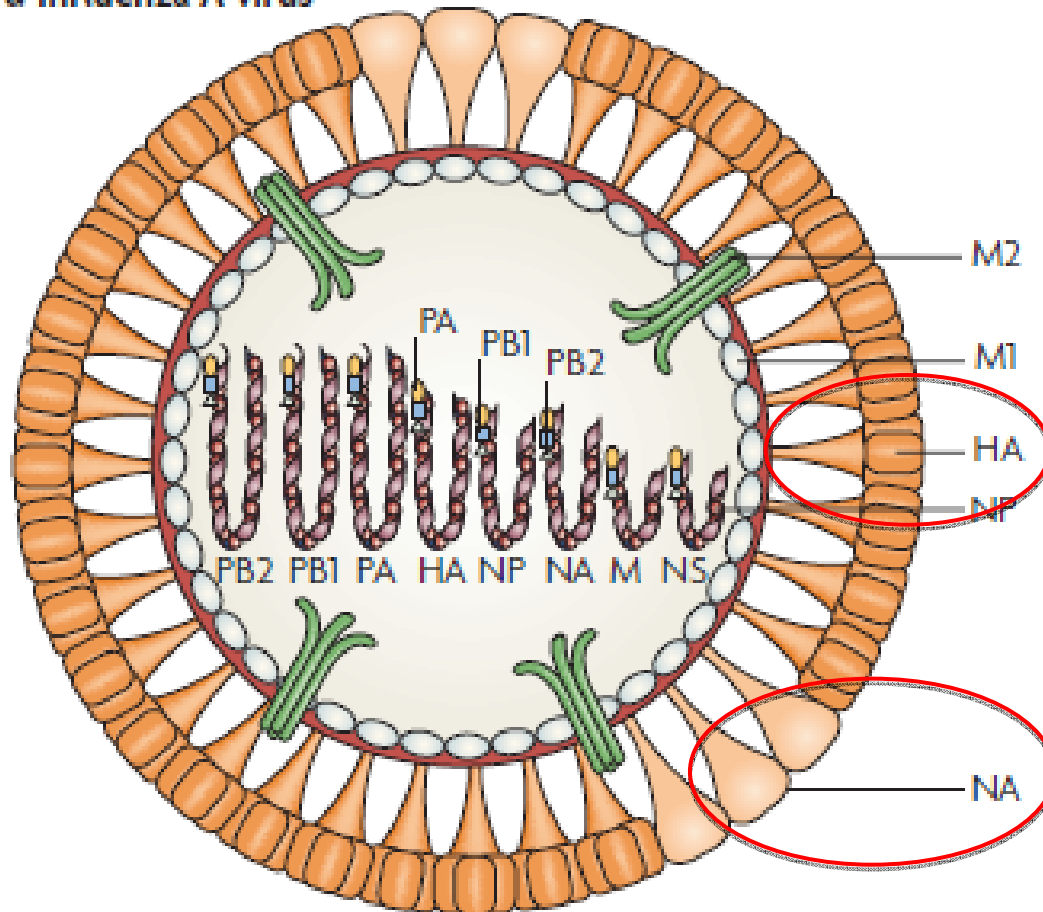


Assessing Immunogenicity of Influenza Vaccines

Nicolas Sabarth
Baxter Bioscience

Influenza - Virus

a Influenza A virus



Subbaro et al 2007

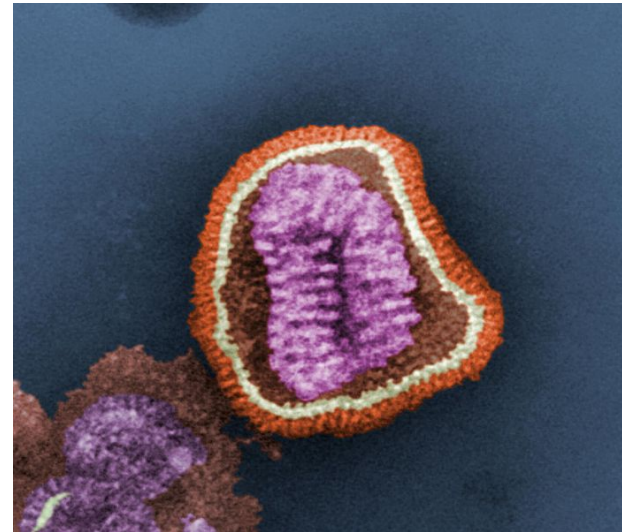
- Proteome: 11 proteins
- 2 surface glycoproteins:
 - **Haemagglutinin (HA)**
 - important for infection
 - bind to sialic acids that are linked to host glycoproteins
 - Type H1-H16
 - **Neuraminidase (NA)**
 - important for virus release from infected cells
 - Type N1-N9

Seasonal Influenza Virus

- Currently circulating in humans
 - H1N1
 - H3N2
 - B type
- **Pre-immunity** ⇒ **low pathogenicity!**



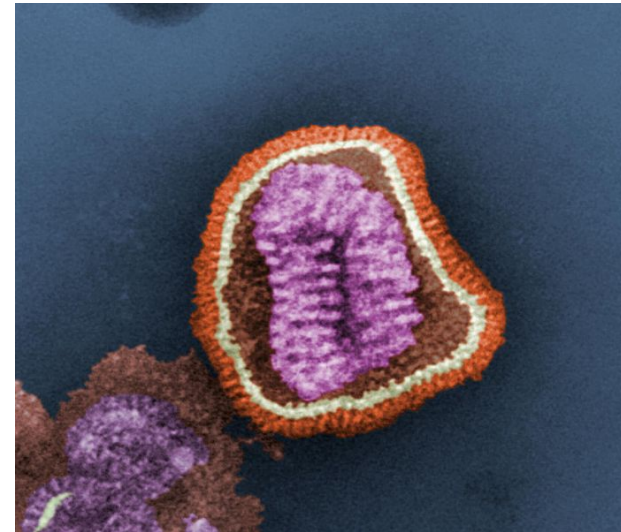
Influenza types



CDC/ Dr. Erskine. L. Palmer; Dr. M. L. Martin

Seasonal Influenza Virus

- Currently circulating in humans
 - H1N1
 - H3N2
 - B type
- } Influenza types
- **Pre-immunity** ⇒ **low pathogenicity!**



CDC/ Dr. Erskine. L. Palmer; Dr. M. L. Martin

Pandemic Influenza Virus

- Emerges when new influenza virus
 1. jumps from animal host to humans
 2. has ability acquired to transmit **human-to-human**
 3. has different **genetic make-up** ⇒ **No pre-immunity** ⇒ potentially **highly pathogenic!**
- Example: **swine flu** - H1N1 (human/swine/avian origin)
- Next **candidates** for pandemic: H9N2, H2N2, **H5N1**, ...

H5N1 influenza („bird‘ flu): The Thread of a Pandemic

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- >600 cases of human infection since 2003
- Mortality rate of ~**60%**
⇒ highly **pathogenic**
- circulating in birds
- So far animal (**bird**)-to-human transmission only (poultry)
→ currently only **thread** of a pandemic
- Adaptation of virus/reassortment ⇒ **human-to-human** transmission ⇒
accelerate virus spread ⇒ **pandemic**

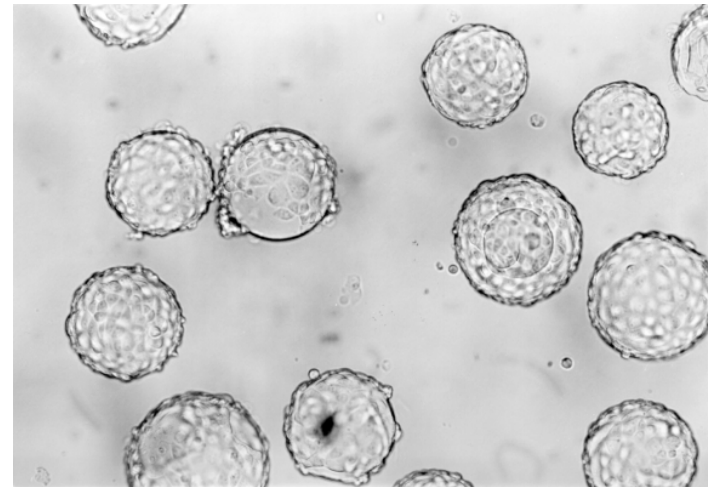


forbesindia

Features

- based on wildtype influenza virus (strain **H5N1 Vietnam/1203/2004**)
- Whole virus vaccine
- Inactivated
- Grown in cell culture (Vero cells)

- Licensed in EU



Correlate of Protection for Influenza Vaccine

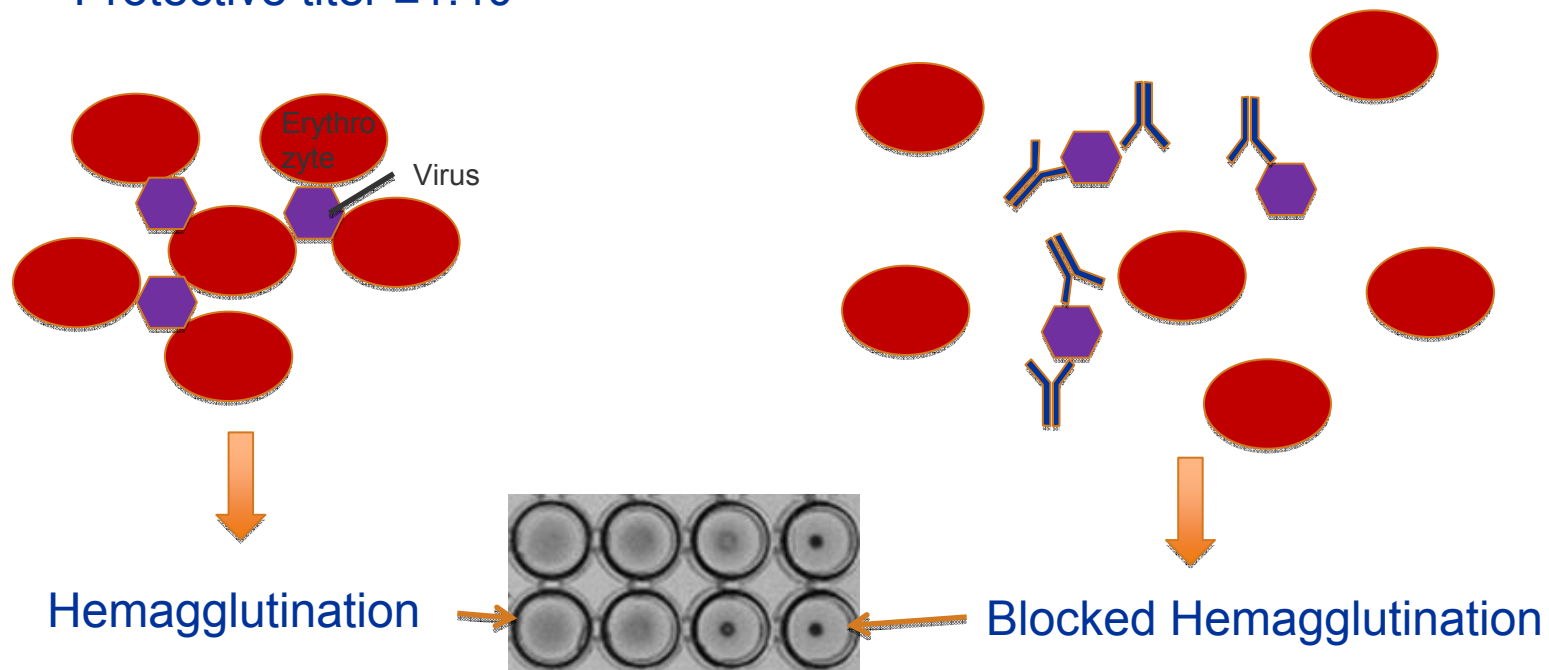
Inactivated/subunit/split influenza vaccine

- Correlate of protection: Antibodies directed against influenza **hemagglutinin (HA)**
- Assessed by
 - **Hemagglutination inhibition assay (HI)**
 - **Single radial hemolysis assay (SRH)**
- Accepted by regulatory authorities (EMA, CPMP/BWP/214/96), based on former challenge studies

Hemagglutinin Inhibition Assay (Palmer, 1975)

• Principle

- Turkey/horse erythrocytes are clotted by hemagglutinin of H5N1 virus which can be inspected by eye
- Anti-HA antibodies block hemagglutination
- Titer = highest serum dilution able to block hemagglutination
- Protective titer $\geq 1:40$

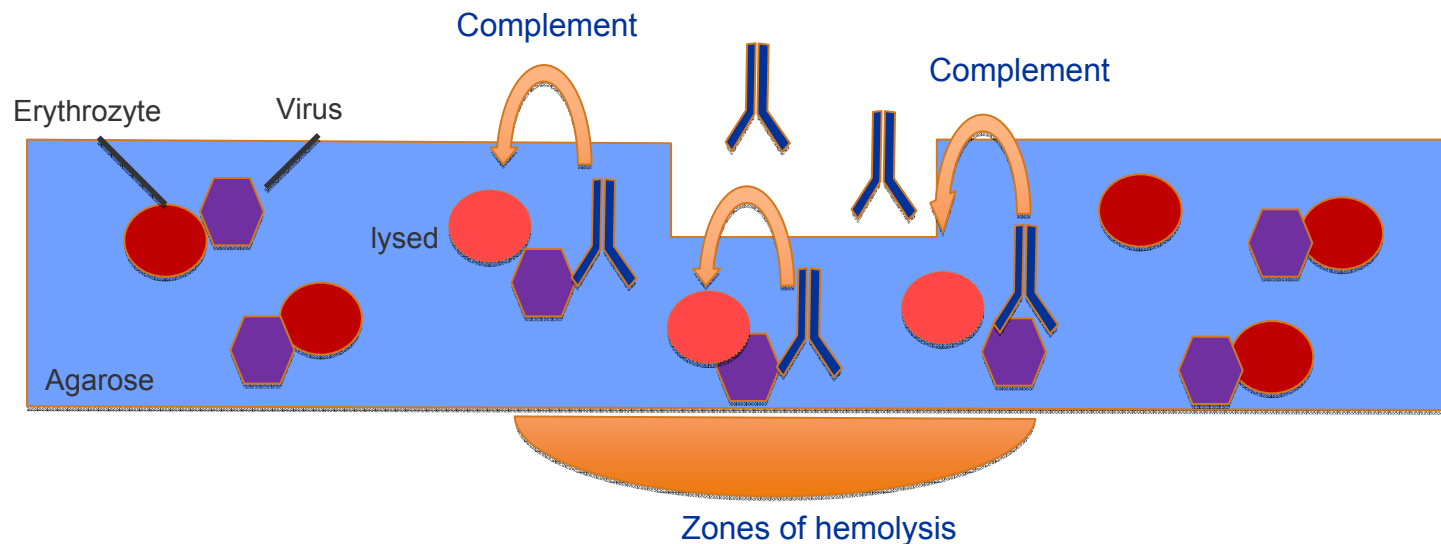


Single Radial Hemolysis Assay (Schild, 1975)

Principle

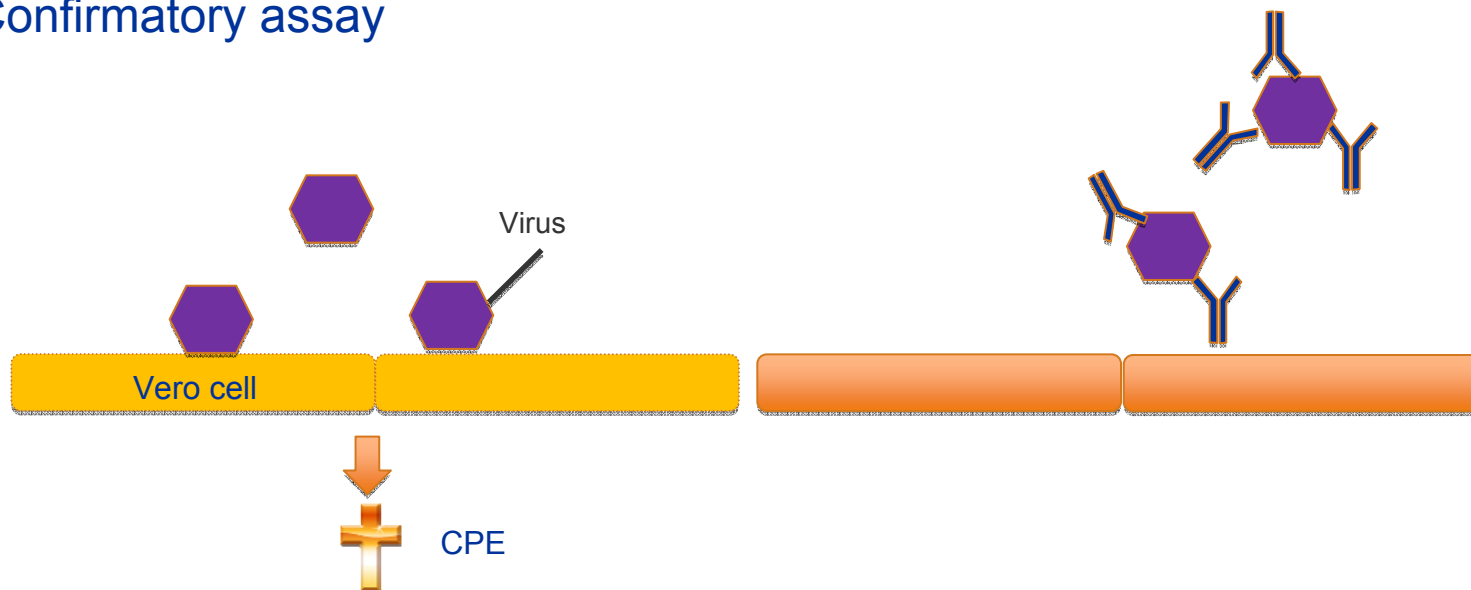
- Sheep/turkey erythrocytes are immobilized in agarose containing virus
- Serum samples diffuse radially from wells punched in the agarose
- Zones of hemolysis are developed by guinea pig complement
- Diameter of hemolysis zone correlates with antibody concentration
- Protective titer $\geq 25\text{mm}^2$

Zones of hemolysis



Virus Neutralization Assay

- **Principle**
 - Confluent layer of Vero cells is infected by virus
 - Infections results in cytopathogenic effect detectable by microscope
- Need for live virus - BSL3+ lab for pandemic strains necessary
- Functional assay
- Works well with **avian** influenza
- Might be more suitable for children than HI or SRH
- Confirmatory assay



Neuraminidase Inhibiting Antibodies

Neuraminidase

- Important for virus release from infected cells

Role of Neuraminidase inhibiting antibodies

- NA-specific antibodies **reduce** the **release of virus** from infected cells
- NA-specific responses **reduce viral spread** and severity of influenza **illness** in animal & human challenge studies
- NA-specific responses reduced the impact of the **1968/69 H3N2 pandemic**

Hence, ...

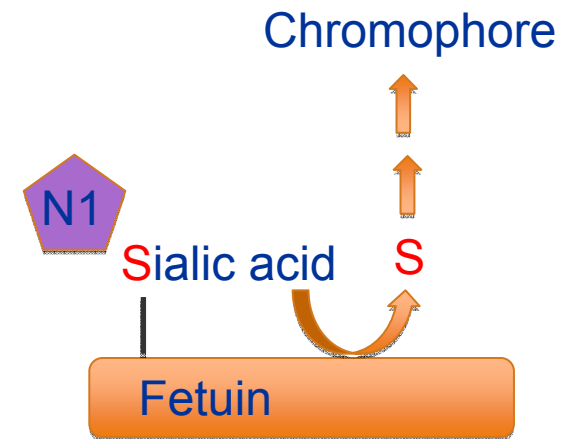
- EMA encourages applicants to measure anti-neuraminidase antibody elicited by mock-up vaccines (EMEA/CPMP/VEG/4717/03-Rev.1)

Thiobarbituric acid based neuraminidase inhibition assay (TBA) (Aymard-Henry, 1973)

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Measurement of neuraminidase activity

- Neuraminidase (of virus) removes sialic acids from substrate fetuin
- Sialic acids oxidized by periodate
- Oxidized sialic acids reacts with thiobarbituric acid, resulting in β -formyl pyruvic acid (chromophore)
- Measure chromophore at 550nm



Neuraminidase inhibition (NI)

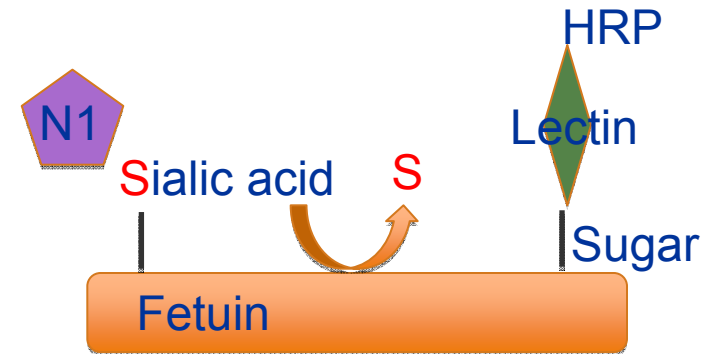
- Inhibition of neuraminidase activity by antibodies → functional assay
- Definition of NI antibody titer: serum dilution inhibiting 50% of neuraminidase activity
- **Validated** Assay (rec neuraminidase VN N1, pos. control: VN N1 specific animal serum)

Enzyme-Linked Lectin Assay (ELLA) Neuraminidase Inhibition Test (Lambre, 1990)

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Measurement of neuraminidase activity

- Neuraminidase removes sialic acids from substrate fetuin
- Sugar residues remain exposed on fetuin
- Sugar residues are detected by peanut Lectin (HRP-labeled)



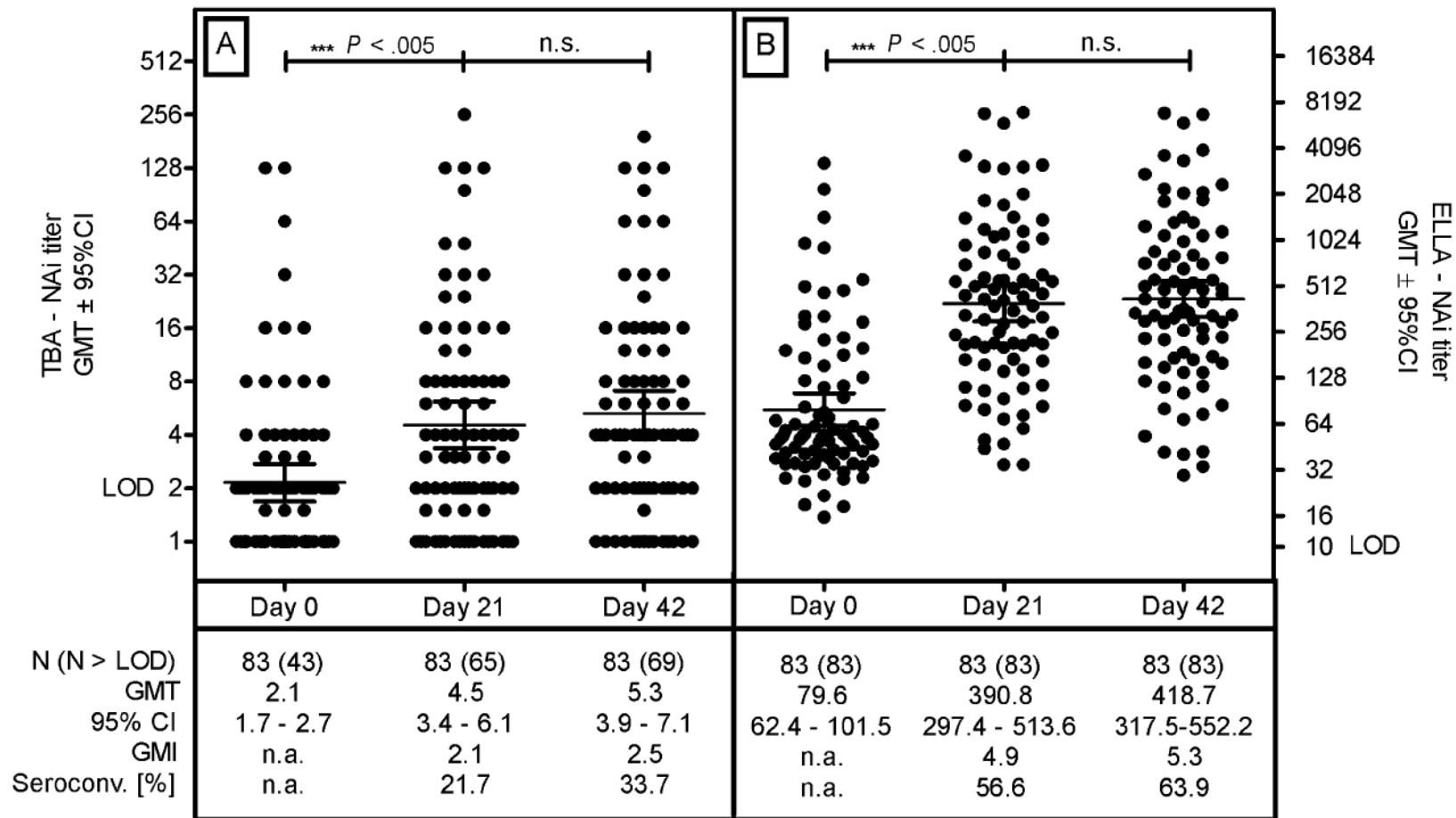
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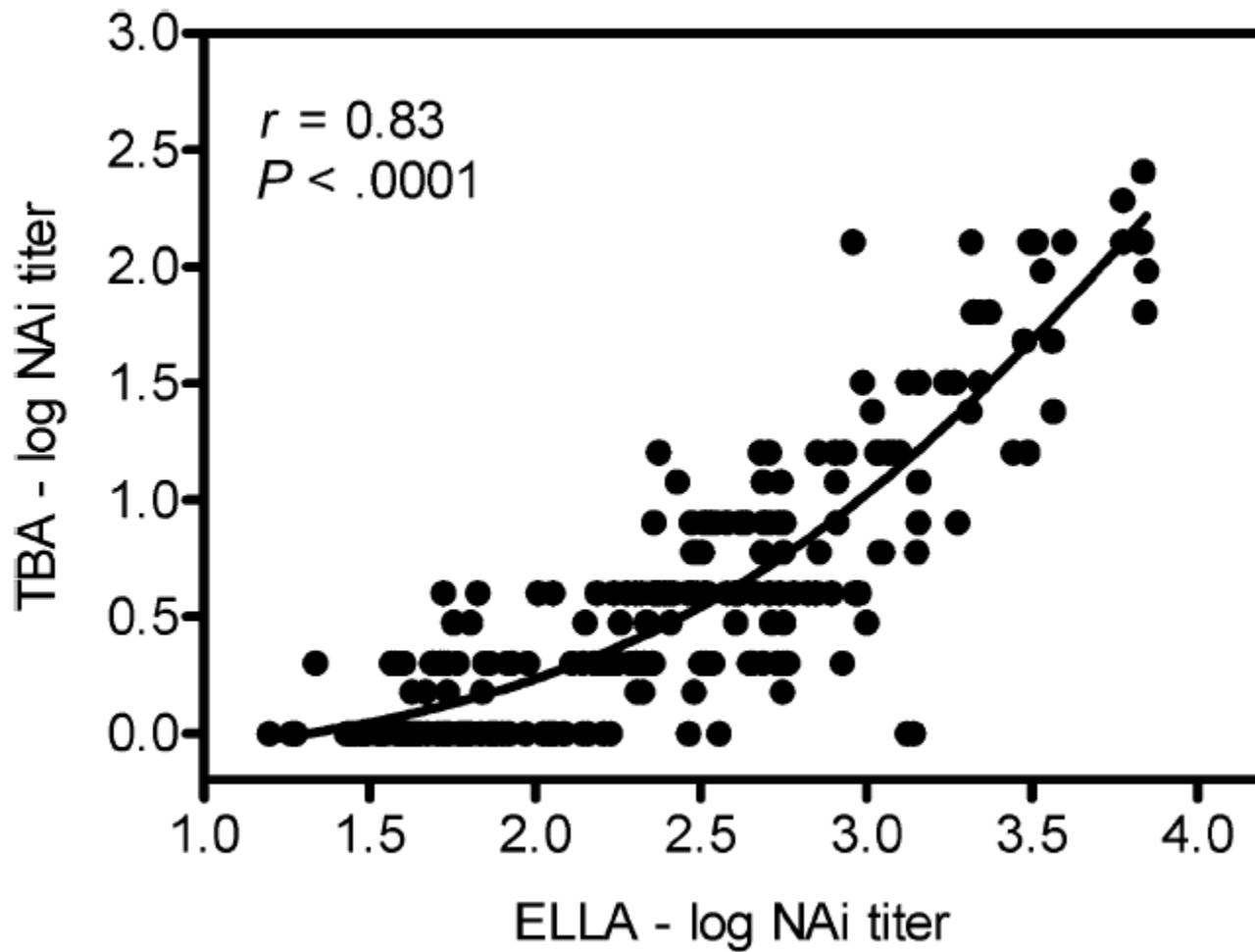
H5N1 vaccination induces NI antibodies

Clinical study (Ehrlich et al., 2008)

- PI/II, healthy volunteers, 18-45 yrs of age
- 7.5 and 15 µg H5N1 vaccine dose groups (n=83), Immunization days 0, 21; Sampling days 0, 21, 42



TBA NI Titer and ELLA NI Titer correlate



Correlation between Antibody Titers by different Assays

	TBA	ELLA	MN	SRH
TBA		$r = 0.83; P < .0001$	$r = 0.52; P < .0001$	$r = 0.38; P < .0001$
ELLA	$r = 0.83; P < .0001$		$r = 0.67; P < .0001$	$r = 0.50; P < .0001$
MN	$r = 0.52; P < .0001$	$r = 0.67; P < .0001$		$r = 0.71; P < .0001$
SRH	$r = 0.38; P < .0001$	$r = 0.50; P < .0001$	$r = 0.71; P < .0001$	

Nonparametric Spearman correlations were calculated

Abbreviations: ELLA, enzyme-linked lectin assay; MN, microneutralization; SRH, single radial hemolysis; TBA, thiobarbituric acid.

Conclusion

- **Correlate of protection for influenza vaccine** are hemagglutinin specific antibodies (HI, SRH assay)
- **Neuraminidase inhibiting antibodies** contribute to protection
- Neuraminidase inhibiting antibodies can monitored by **TBA** and **ELLA** assay

- **Baxter's** whole virus pandemic **H5N1 vaccine** is equally effective in
 - inducing **antibodies to hemagglutinin** (Ehrlich et al., 2008)
 - inducing **antibodies to neuraminidase** (Fritz et al., 2011)

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