Assays to assess antibody responses to influenza neuraminidase

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Influenza Neuraminidase

- NA contributes to influenza life cycle in several ways
  - facilitates traffic of virus to the respiratory epithelium (Mastrosovich et al., 2004)
  - allows virus release from infected cells (Palese et al; Griffin and Compans)
  - prevents virus aggregation
  - Contributes to aerosol transmissibility
- NA inhibitors are effective antivirals
- Antibodies that inhibit NA activity reduce disease symptoms and duration of infection

NA inhibition assays tested/optimized/established at CBER

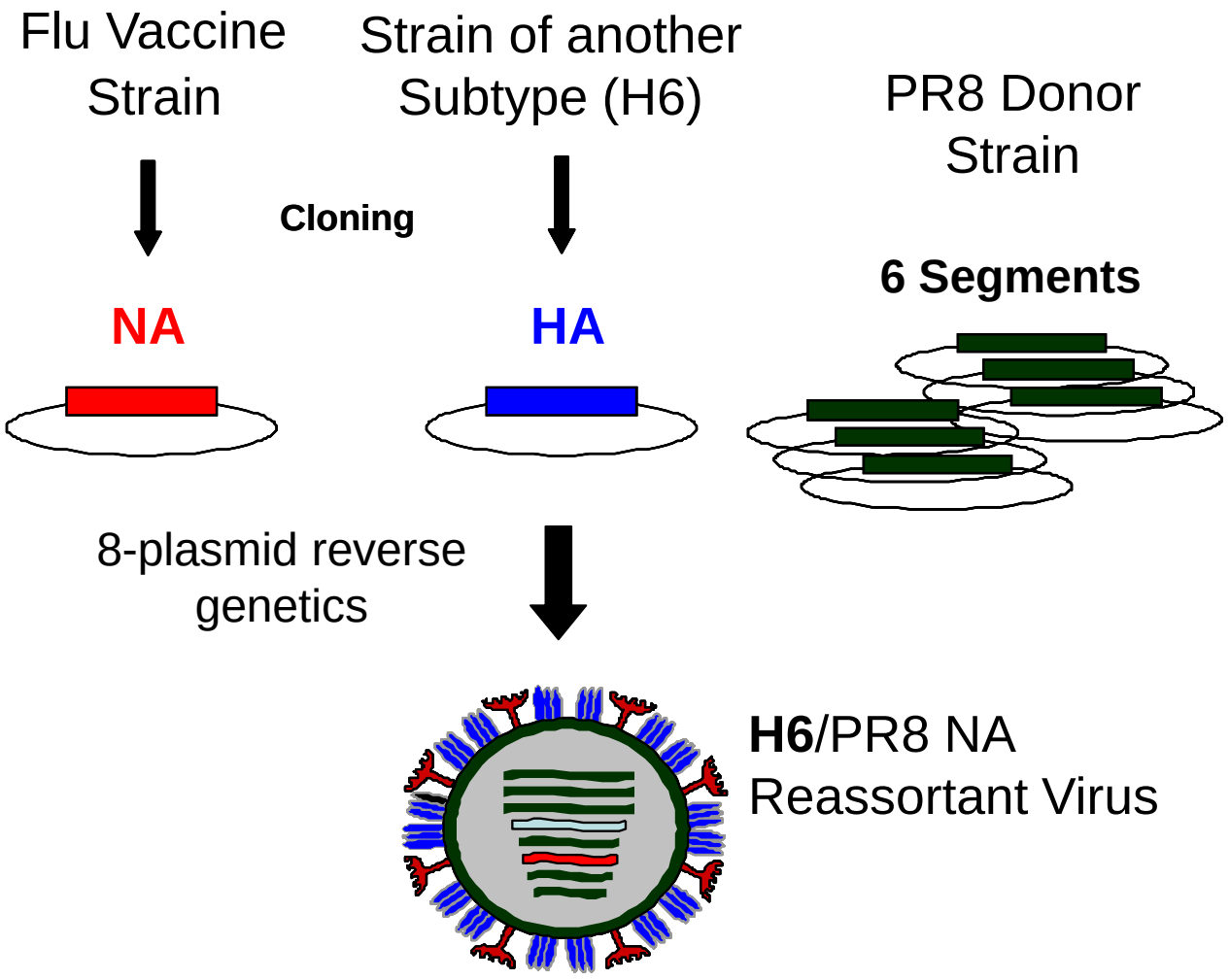
- ELISA
  - *Not functional; difficult to ensure native structure of NA on plate*
- Plaque size reduction assay
  - *Kilbourne et al., 1968, Compans et al., 1969*
- Warren-Aminoff thiobarbituric acid (TBA) method
  - *Chemical conversion of sialic acid to chromophore*
  - *Webster et al., 1968*
  - *Miniaturized to run larger numbers of samples (Sandbulte et al., 2009)*
- Enzyme-linked lectin assay
  - *Lambre et al., 1986*
  - *Greater throughput than TBA assay; does not use harmful chemicals*
  - *Used by most laboratories measuring NI titers*
- NA-specific neutralization assay (AVINA)
  - *Similar to CDC microneut assay, however read-out is NA activity*
  - *Contribution of both HA-specific and NA inhibiting antibodies to change in signal*
- Single step assays using labeled substrates

Elements to ensure accuracy of NI assay

- **Substrate**
  - Should mimic ‘bulk’ of natural substrate
    - Fetuin
    - Cell surface glycoproteins
    - Synthesis of labeled large substrate

- **Source of NA**
  - Purified NA
  - Whole virus
    - mismatched HA
    - detergent disrupted virus

Generation of virus reagents with strain-specific NA



Enzyme-Linked Lectin Assay (ELLA)

Peanut agglutinin (PNA) binds to residual terminal galactose

Lambre et al, 1990

Bob Couch
Cate et al., Vaccine 2010

Overview of ELLA to determine NI titers (1)

1. Coat plates with fetuin

Protein —— Sugars —— Galactose —— Sialic acid

2. Add in standard amount of virus/NA with sera dilutions

NA

Protein —— Sugars —— Galactose —— Sialic acid

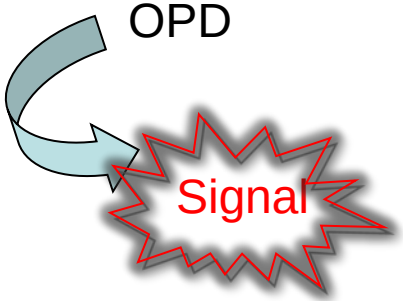
Protein —— Sugars —— Galactose

3. Add in PNA-HRPO

Protein —— Sugars —— Galactose::PNA-HRPO

4. Add in substrate (OPD)

Protein —— Sugars —— Galactose::PNA-HRPO



Overview of ELLA to determine NI titers (2)

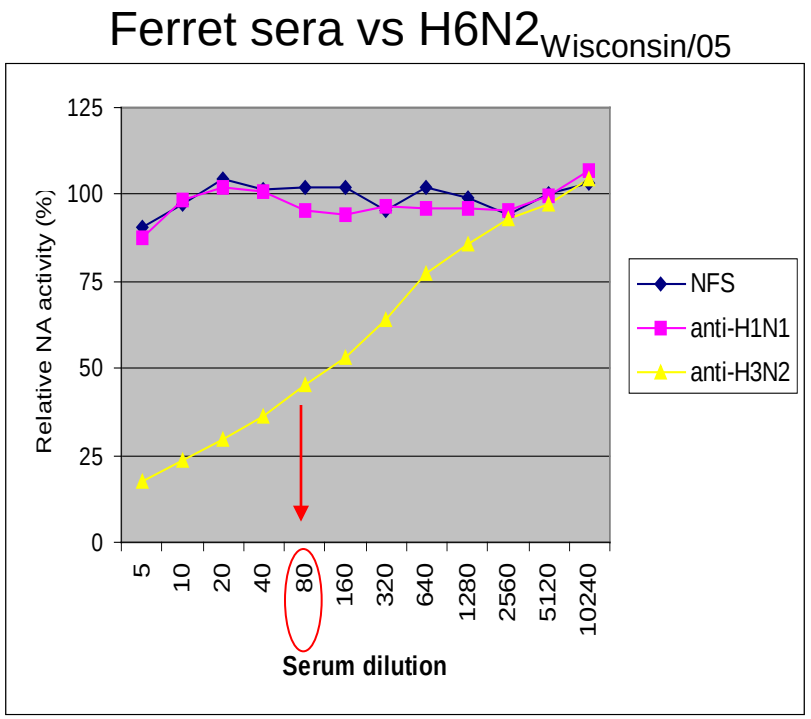
Sample preparation

Animal and human sera inhibit NA activity non-specifically

- Heat-treatment (56 ºC, 1 hour)
- Freeze-thawing for limited number of times can help but generally is not necessary
- RDE-treatment followed by heat-inactivation may be necessary in some cases

Determination of NI titer

- End-point analysis: highest serum dilution able to inhibit 50% of NA activity
- 50% inhibition analysis: nonlinear regression to calculate titer



Enzyme inhibition assays are performed to distinguish NA subtypes and heterologous viruses within a subtype

Antigenic Differences between Heterologous N1's

	NI titer of ferret antiserum against			
NA antigen	A/HK/8/68	A/BR/59/07	A/CA/04/09	A/VN/1203/04
A/HK/8/68 (H3N2)	1280	<5	<5	<5
A/BR/59/07 (H1N1)	<5	640	10	<5
A/CA/04/09 (H1N1pdm)	<5	40	1280	10
A/VN/1203/04 (H5N1)	<5	40	20	320

There is diversity within human seasonal viruses due to antigenic drift

Antigenic differences between seasonal N1's

	NI titer of ferret antiserum against			
NA antigen	A/TX/91	A/NC/99	A/SI/06	A/BR/07
A/TX/91 (H1N1)	320	2560	1280	320
A/NC/99 (H1N1)	160	1280	1280	160
A/SI/06 (H1N1)	160	1280	1280	160
A/BR/07 (H1N1)	<5	80	80	640

Sandbulte et al., PNAS 2011

Next steps

- Validate method for preparing B antigens for NI assays

- Discriminate between strain-specific and broadly-reactive NA antibodies
  - *Adsorption*
  - *Use antigens that have conserved epitopes mutated*

- Interlaboratory study to evaluate assay reproducibility
  - Same method used by at least 4 laboratories (CBER, CDC, Erasmus, Focus Diagnostics)
    - » additional labs?
    - » Labs that use slightly different steps could perform analysis using their own protocol in parallel with ‘standard’ procedure
  - NI titers for at least one N1 and one N2 antigen
    - » *BPL-inactivated H6N1 and H6N2 reassortants distributed*
    - » *20 samples to include 14 human sera (some added as blind repeats), 4 ferret sera (including serum from naïve ferret), 2 monoclonal antibodies (N1 and N2 standard)*

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