Viral Neuraminidase

1. Assays to measure NA Inhibition Antibody Titers

2. Assay that uses NA as an end-point to measure virus neutralization titers

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Disclaimer: the ideas presented are those of the author and not official recommendations of the FDA

Antibodies that inhibit NA activity contribute to protection from disease

- NA-specific antibodies reduce the yield and release of virus from infected cells, resulting in reduced plaque size
  - Kilbourne et al., 1968, Compans et al., 1969
- NA-specific responses reduce viral replication and prevent disease
  - In humans (Couch et al., 1974; Kilbourne et al., 1975; Ogra et al., 1977; Beutner et al., 1979; Clements et al., 1989)
  - In mice (Schulman et al., 1968; Johansson et al., 1987; etc)
- NA-specific responses reduced the impact of 1968 H3N2 pandemic
  - Eickhoff and Meiklejohn, 1969; Monto and Kendal, 1973
- NA-specific responses protect against lethal doses of highly pathogenic avian influenza
  - In chickens (Rott et al., 1974; Webster et al., 1988; Sylte et al., 2007)
  - In mice (Sandbulte et al., 2007)

Neuraminidase Inhibition Assay

"Traditional" thiobarbiturate assay (TBA):

(Warren, 1959; Webster and Laver, 1967; WHO: Methods for Influenza surveillance)

- not designed for high throughput
- reproducible within lab (Hennessy and Davenport, 1972)

Miniaturization of TBA

Matt Sandbulte

- Objective is to analyze large numbers of samples
- Replace glass tubes with PCR tube strips/96-well plates
- Instead of boiling samples in water bath, heat in thermocycler
- Instead of reading absorbance in cuvettes use plate reader

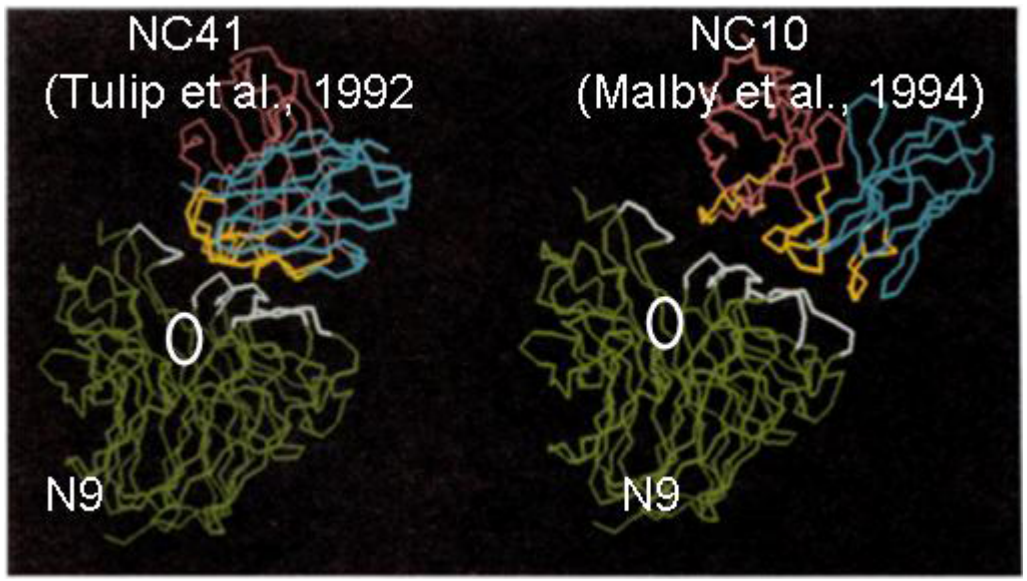
Points to consider in measuring NI titers

Choice of virus – antigenic hybrid virus

Need for reference antisera

Choice of substrate – fetuin instead of small substrates (NANL or MU-NANA)

Webster et al., 1987: identified several monoclonal antibodies that inhibit enzymatic activity with large (fetuin) but not small (NANL) substrates



Alternative NA assays

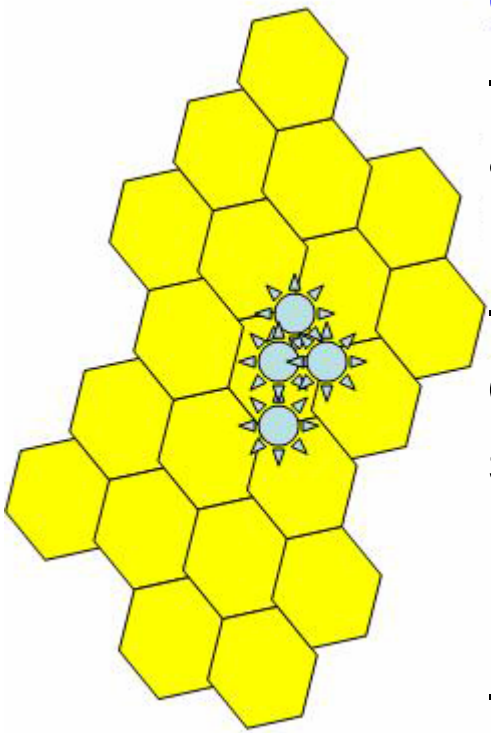
- Hemagglutination inhibition:
  - Steric hindrance by NA-specific antibodies will inhibit virus with mismatched HA (Fedson et al., 1971)

- Lectins:
  - PNA-peroxidase (Lambre et al., 1990) or RCA-biotin (Onodera, 1994)
  - Plates are coated with substrate (fetuin); after incubation with virus, instead of quantitation of released sialic acid, the exposed Gal or Lactose moeities bind to PNA or RCA.

- Synthesis of large labeled substrates

Assay sensitivity? robustness? validation?

Development of HTS assay to measure neutralizing antibody responses that include NI activity



Cell-based virus replication assays

- Plaque reduction neutralization assay (number and size of plaque)

-Microneutralization assay: ELISA-based detection, with anti-NP antibody followed by secondary HRP-conjugated antibody (24 hr post infection)

-High throughput screening assay with NA used to quantitate virus (20 hr post-infection)

NA activity is a hallmark of influenza A, B viruses

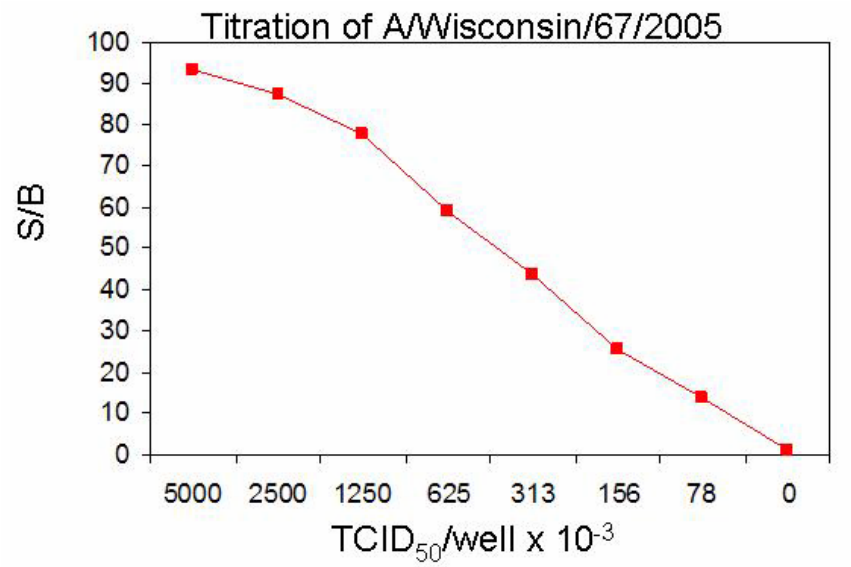
(Gottschalk, 1957)

basis of rapid diagnostic tests (Yolken et al., 1980; ZstatFlu rapid test)

2-(4-methylumbelliferyl)-*a*- -*N*-acetylneuraminic acid ⟶ NANA + MU

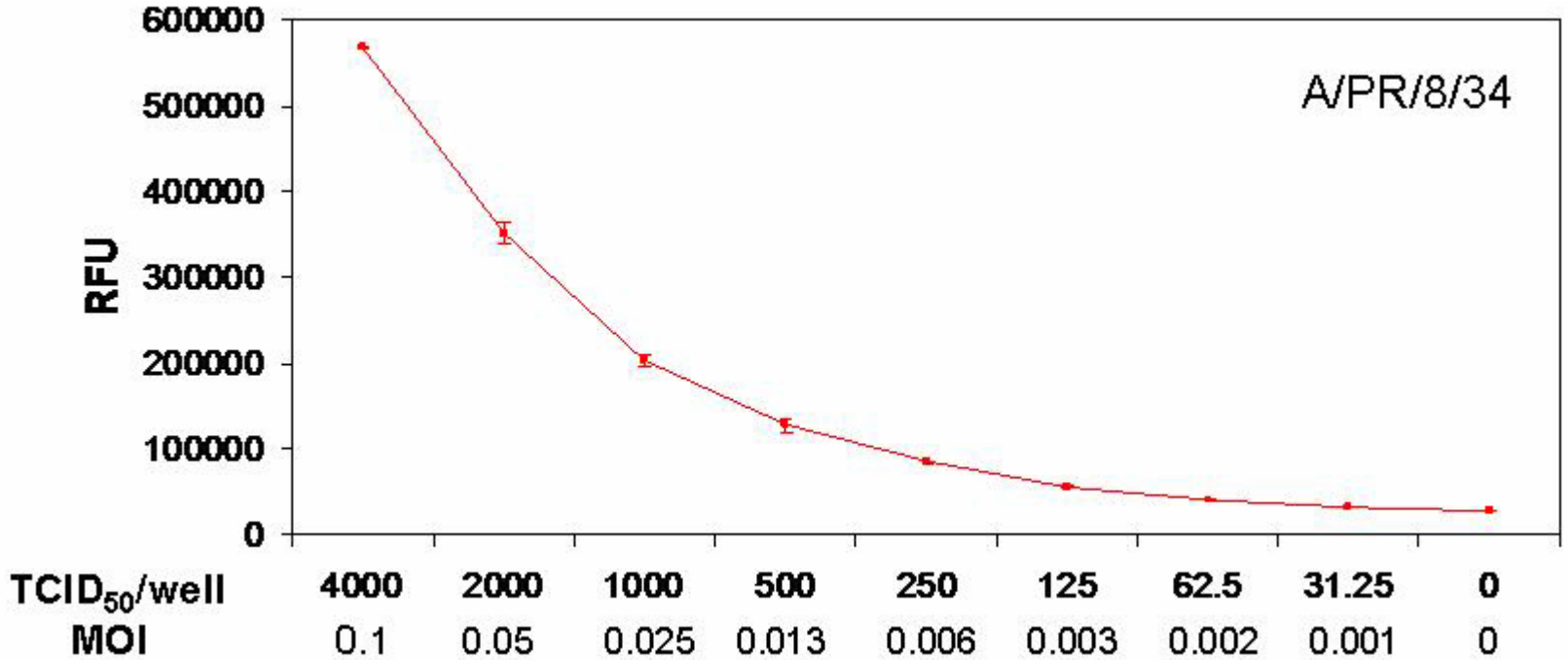
(Potier et al., 1979)

NA activity is proportional to amount of virus



NA activity is a sensitive, early means to identify influenza replication

(Pachucki and Creticos, 1988)



AVINA (Accelerated Viral Inhibition-NA) assay

Viral culture

1. Prepare serum (antiviral) dilutions and controls
2. Add virus/TPCK-trypsin/BSA
3. Add washed cells
4. Incubate at 37 °C for 20 hr
5. Remove 25 µl supernatant for NA assay

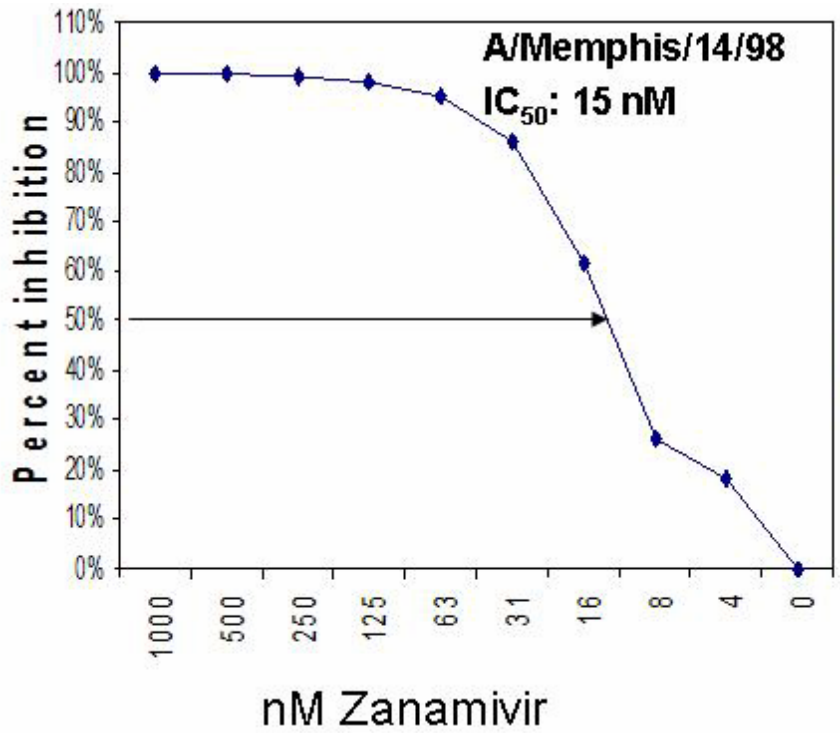
NA assay

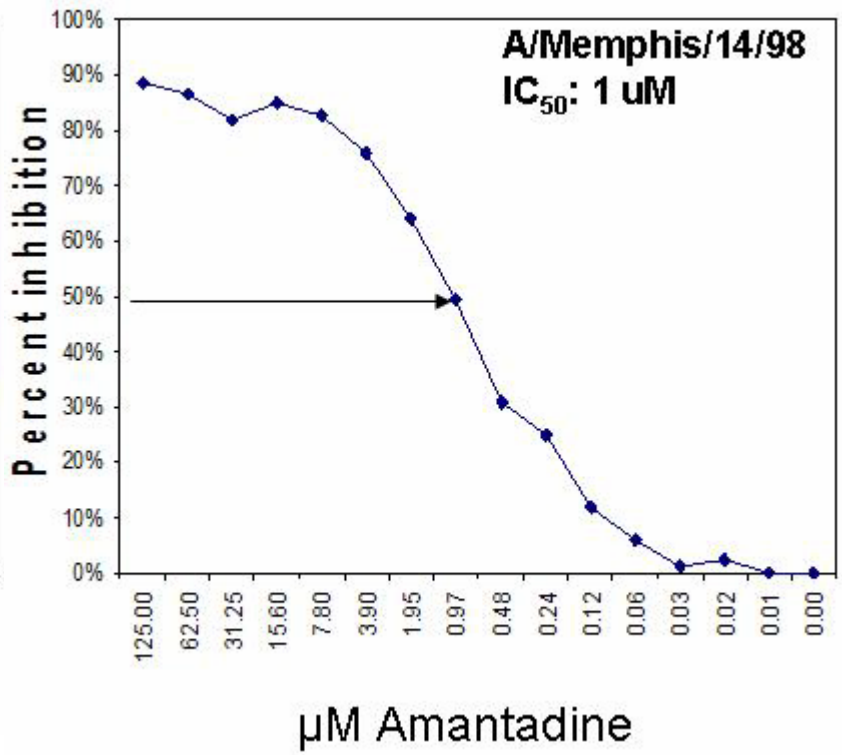
1. Add substrate to supernatant
2. Incubate 60 min at 37 °C
3. Stop reaction
4. Read fluorescence (0.1 sec per well).
5. Calculate 50% inhibition end point

Applications of AVINA

Arash Hassantoufighi
Johns Hopkins University: Min Li, Meng Wu

- identify antiviral activity
- determine IC_{50}(antiviral resistant strains)
- determine neutralizing antibody titers





Comparison of Microneut and AVINA

	A/New Caledonia/20/99		A/Wisconsin/67/2005	
	microneut	AVINA	microneut	AVINA
Sheep control	<200	<200	<200	<200
Sheep α-Wisconsin	<200	<200	2,616	2,901
Sheep α-N-Caledonia	2,911	11,700	<200	<200
Human serum 1-pre	144	250	111	200
Human serum 1-post	800	4,366	889	1,448
Human serum 2-pre	791	2,560	220	688
Human serum 2-post	803	4,732	225	1,383

Conclusions

- NI titers should be measured routinely in vaccine trials, particularly in pandemic vaccine trials.

- A practical method that allows screening of large numbers of serum samples for NA inhibition titers will be a step toward demonstrating the breadth of antibody specificity following infection and vaccination. Such a test needs to be sensitive, practical, and robust.

- NA provides a marker that can be used to follow virus replication, providing a sensitive and reproducible method that is likely to capture NI antibody titers in addition to HA-specific neutralizing antibody titers.