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Towards an Improved Wild-type Sequence Based Hemagglutination Inhibition Assay (HAI) for the Evaluation of Influenza Vaccines: Challenges and New Developments

Vivek Shinde, Bin Zhou, Mike Massare, MingZhu Zhu, Joyce Plested, Gale Smith, Lou Fries, Greg Glenn

NASDAQ:NVAX

Outline

- **The classical hemagglutination inhibition (c-HAI) assay:** background and current problems
- **The w*ild-type* VLP hemagglutination inhibition (wt-HAI) assay:** a potential way forward
- **Assessing performance of wt-HAI vs c-HAI**

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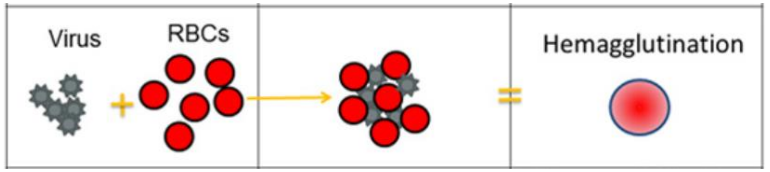
The background:
Two principles form the mechanistic basis of the classical hemagglutination inhibition (c-HAI) assay

1. The hemagglutinating property of influenza viruses
   - Mediated by interactions between the hemagglutinin (HA) head receptor binding domain (RBD) and the terminally sialylated glycans (sialic acid receptors) on the surface of red blood cells (RBCs)



2. The ability of influenza virus-specific anti-HA antibodies, whether infection- or vaccine-derived, to interrupt this interaction, and thereby inhibit hemagglutination

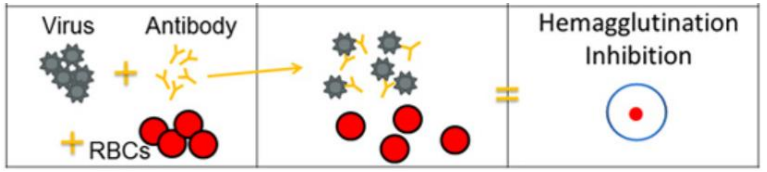


Image adapted from: https://www.cdc.gov/flu/about/professionals/antigenic.htm

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The background:
The classical hemagglutination inhibition (c-HAI) assay has been in widespread use because of its utility

- Historically, the assay achieved “reference” status as a serological tool
  - “…the HAI test remains the assay of choice for global influenza surveillance and for determining the antigenic characteristics of influenza viral isolates…” – *WHO manual 2011*
  - “For the purposes of accelerated approval.., the HI antibody response may be an acceptable surrogate marker of activity that is reasonably likely to predict clinical benefit.” – *FDA Guidance MAY 2007*

- Applications have included:
  - Global influenza surveillance, vaccine strain selection, and determination of antigenic relatedness (match)
  - Assessment of influenza vaccine immunogenicity
  - Establishment of immunologic correlates of clinical risk/protection
  - Establishment of surrogate (immunologic) endpoints for licensure of influenza vaccines
  - Population based seroepidemiological studies of seasonal and pandemic influenza virus infection and immunity
  - Serological diagnosis of influenza virus infection, including asymptomatic infection
  - Screening for influenza “naïve” volunteers in human influenza virus challenge studies

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The problem(s):

Three critical problems threaten the utility and credibility of the classical hemagglutination inhibition (c-HAI) assay

1. **Impaired performance against contemporary A/H3N2s**: limited ability to measure HAI antibody responses to contemporary A/H3N2 viruses
   - Reduced binding to avian RBCs glycans due to evolutionary changes in receptor specificity
   - A/H3N2 viruses to fail to agglutinate avian RBCs, rendering c-HAI unworkable

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2. **Reduced clinical relevance:** reliance on egg-derived HA antigens may yield biased results with unclear clinical meaning
   - **Egg-adaptive antigenic changes** have been reported to **degrade the effectiveness** of egg-derived influenza vaccines[1,2]
   - Egg-adaptive changes are **equally problematic for egg-derived HA antigens used in c-HAI assays** to evaluate these vaccines[2,3]
   - This introduces **egg-adaptation bias that favors detection of HAI antibodies directed at egg-adapted viruses/vaccines**
   - Creates **a disconnect between** the high titered **c-HAI antibody responses and** increasingly **poor field vaccine effectiveness.**

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   - This introduces **egg-adaptation bias that favors detection of HAI antibodies directed at egg-adapted viruses/vaccines**
   - Creates **a disconnect between** the high titered **c-HAI antibody responses and** increasingly **poor field vaccine effectiveness.**
3. **Unintended consequence:** creates barriers to entry for next-gen recombinant vaccine technologies
   - The serological performance of new, non-egg-derived, *wild-type* HA-targeting vaccine technologies may be systematically underestimated to egg-derived conventional products.

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A potential way forward:

We developed a wild-type VLP hemagglutination inhibition assay (wt-HAI) to overcome critical problems with c-HAI

What are the principle differences in the WT-HAI vs. C-HAI assays?

	wt-HAI	c-HAI
Agglutinating particle	Recombinant virus-like-particles (VLPs) expressing ***wild-type*** HA and NA antigens	Egg-derived/adapted HA antigen/virus
Indicator particle	Human type-O red blood cells (RBCs)	Typically avian (chicken or turkey) or guinea pig red blood cells (RBCs)

- These assay modifications **rehabilitate HAI assay performance** and **restore its clinical relevance** as a serological tool
  - Restores agglutination of contemporary A/H3N2 viruses to RBCs
  - Restores the ability to interrogate clinically relevant HAI antibody responses to circulating ***wild-type*** H3N2 viruses

- We assessed performance of the assay in several ways:
  - Evaluated the agglutinating potential of various combinations of agglutinating particle (virus or VLP) and indicator particles (species of RBCs)
  - Compared performance of wt-HAI vs. c-HAI antigen reagents against US CDC ferret reference antisera
  - Compared performance of wt-HAI and c-HAI against microneutralization (MN) assay using sera from a recent phase 2 trial

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Assessment of agglutinating potential: various combinations of RBCs and cell-passaged viruses or VLPs

For A/H3N2 viruses, combination of VLPs and human RBCs yielded significantly better hemagglutination titers

HA Sequence Source	Agglutinating Particle	HA Titer (µ/mL) with indicated RBC specie		
		Turkey RBC	Guinea Pig RBC	Human RBC
A/Singapore/INFIMH/16-0019/16 (H3)	VLP	**128**	**1,024**	**32,768**
A/Singapore/INFIMH/16-0019/16 (H3)	MDCK virus	64	32	64
A/Hong Kong/4801/14 (H3)	VLP	**256**	**2,048**	**65,536**
A/Hong Kong/4801/14 (H3)	MDCK virus	64	64	128
A/Switzerland/97/15293/13 (H3)	VLP	**2,048**	**2,048**	**8,192**
A/Switzerland/97/15293/13 (H3)	MDCK virus	256	64	128
A/Texas/50/12 (H3)	VLP	**8,192**	**8,192**	**32,768**
A/Texas/50/12 (H3)	MDCK virus	16	16	128

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Contemporary wild-type A/H3N2 viruses poorly agglutinated turkey or guinea pig RBCs

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		Turkey RBC	Guinea Pig RBC	Human RBC
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A/Singapore/INFIMH/16-0019/16 (H3)	MDCK virus	64	32	64
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Wild-type VLPs **improved** agglutination compared to virus, particularly with guinea pig RBCs

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A **combination** of human type-O RBCs and wild-type VLPs **dramatically improved** agglutination

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Assessment of agglutinating potential: various combinations of RBCs and cell-passaged viruses or VLPs

For A/H1N1 and B viruses, VLPs are more important than choice of RBC species in improving agglutination

For A/H1N1 and B viruses, wild-type VLPs improved agglutination more so than did choice of species of RBC

HA Sequence Source	Agglutinating Particle	HA Titer (µ/mL) with indicated RBC specie		
		Turkey RBC	Guinea Pig RBC	Human RBC
A/Michigan/45/15 (H1N1)	VLP	16,384	8,192	32,768
A/Michigan/45/15 (H1N1)	MDCK virus	64	32	128
B/Brisbane/60/08 (Victoria)	VLP	16,384	8,192	32,768
B/Brisbane/60/08 (Victoria)	MDCK virus	64	64	512

Comparison of agglutinins: wt-HAI (wt-VLP) vs. c-HAI (egg- or cell-passaged virus) against reference antisera

wt-HAI (with wt-HA VLPs) yielded titers closely matching c-HAI (with egg-/cell-virus) against historical reference antisera

- **Next**, we HAI tested CDC historical reference ferret antisera, to compare the performance of classical antigens (cell- or egg-derived viruses) vs. counterpart wild-type VLP HA antigens, as the agglutinin
- **Importantly**, none of these historical cell-/egg- derived viruses were known to have undergone the egg-adaptive K160T HA mutation which is now common

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- **Importantly**, none of these historical cell-/egg- derived viruses were known to have undergone the egg-adaptive K160T HA mutation which is now common
- **RESULT:** wt-HAI (using wild-type VLP antigens) yielded titers which closely matched c-HAI (using egg- or cell-grown antigens), with no homologous virus titer differences greater than 2-fold (i.e. the expected variability of the assay)

CDC Reference Ferret Antiserum	Antiserum Lot #	Virus or VLP (HI antigens) Name and Lot #	Passage	HAI Titer	Virus Subtype
Ferret antisera raised to A/Switzerland/97/15293/2013 (H3N2)	63310401 (Egg)	A/South Australia/55/14/E4 36'C 10e6 29Sep15	Egg virus	640	H3N2 (A/Switz-like)
		A/Switz/2013 cell MD-2 10e4 12/24/17	wt virus	160	H3N2
		A/Switz VLP BV1660 20Mar15	VLP wt	320	H3N2
		A/Texas/50/2012 E-8 10e6-6.5 pool 7/16/13	Egg virus	320	H3N2
		A/Texas/50/12 MD-4 10e3 1/29/18	wt virus	160	H3N2
		A/Texas VLP BV1324 Lot 18Dec17	VLP wt	320	H3N2
Ferret antisera raised to A/Texas/50/2012 (H3N2)	61852805 (Cell)	A/South Australia/55/14/E4 36'C 10e6 29Sep15	Egg virus	320	H3N2 (A/Switz-like)
		A/Switz/2013 cell MD-2 10e4 12/24/17	wt virus	320	H3N2
		A/Switz VLP BV1660 20Mar15	VLP wt	160	H3N2
		A/Texas/50/2012 E-8 10e6-6.5 pool 7/16/13	Egg virus	1280	H3N2
		A/Texas/50/12 MD-4 10e3 1/29/18	wt virus	640	H3N2
		A/Texas VLP BV1324 Lot 18Dec17	VLP wt	1280	H3N2

Assessment of validity: can MN (wt-virus) vs. MN (egg-virus) reproduce the differences seen in wt-HAI vs. c-HAI?

Validity of wt-HAI confirmed by MN (wt-virus); egg-derived reagents yield results biased towards egg-derived vaccines

- **Next**, we tested Day 0 (baseline) and Day 28 (post-vaccination) sera from a recent phase 2 vaccine RCT of 1375 older adults
  - We compared the immunogenicity of a recombinant saponin-adjuvanted nanoparticle vaccine (“NanoFlu”) vs. Fluzone High Dose (Fluzone HD)
  - The A/H3N2 component in both vaccines was A/Singapore
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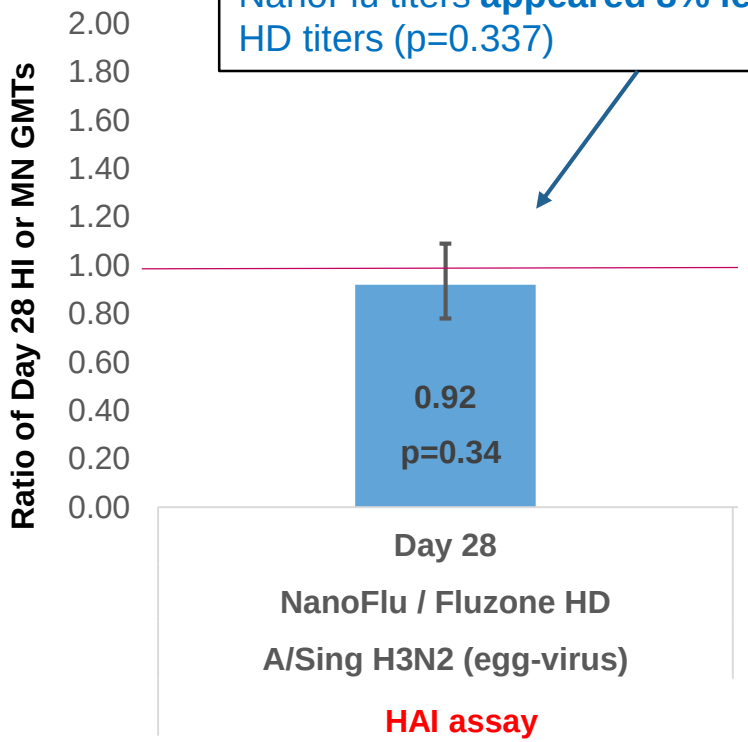
With ***egg-adapted virus reagents*** on c-HAI, NanoFlu titers **appeared 8% less** than Fluzone HD titers (p=0.337)



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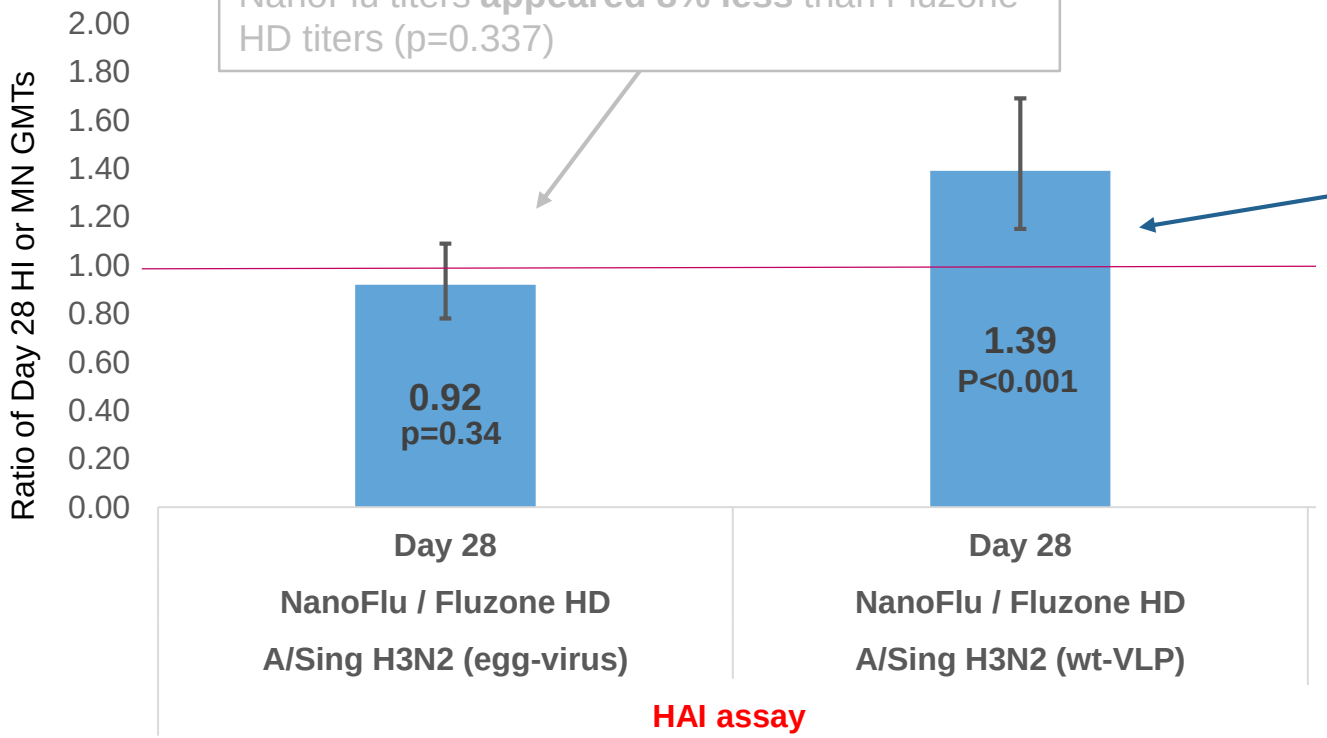

However, with ***wild-type VLP reagents*** on wt-HAI, NanoFlu titers **then appeared 39% greater** than Fluzone HD titers (p<.001)

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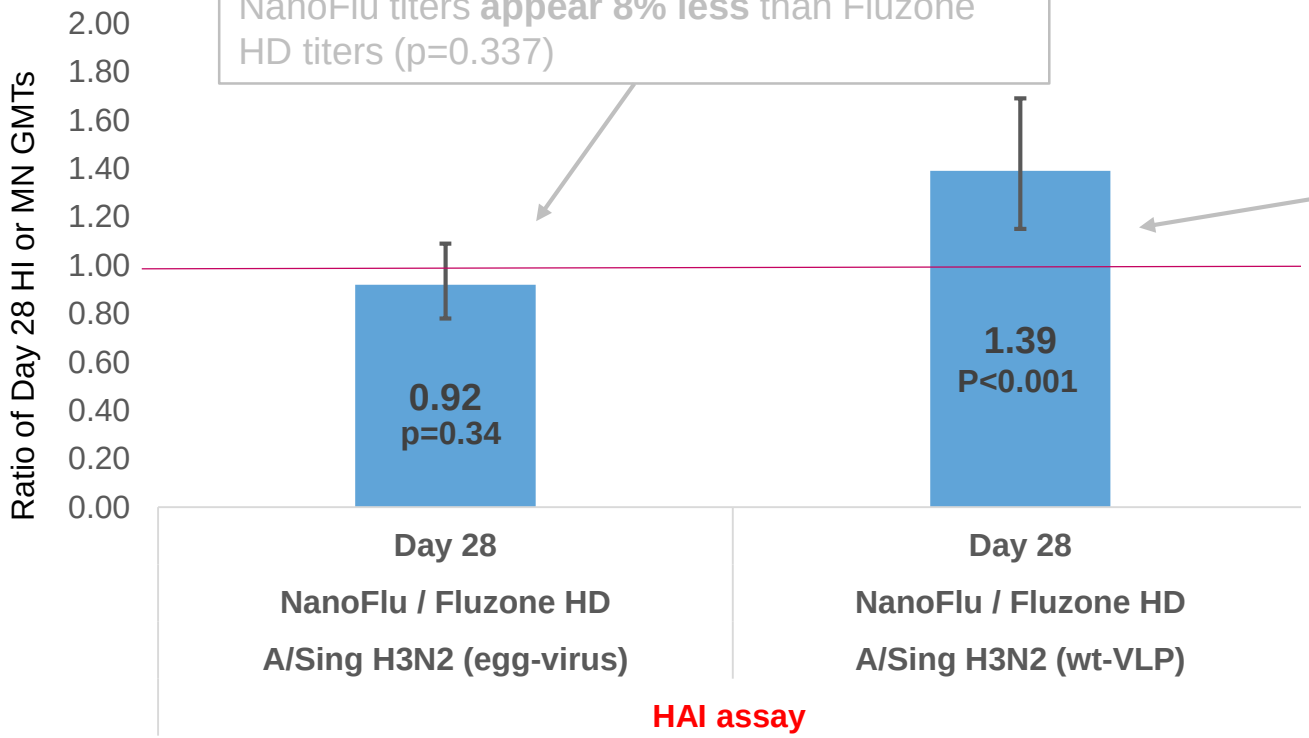

QUESTION:
Could the MN antibody assay confirm the validity of the wt-VLP HAI system, by recapitulating differences seen with egg- vs wt-VLP HAI reagents?

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Use of ***egg-adapted* reagents (virus) on MN** recapitulated results of ***egg-adapted virus* reagents on c-HAI,** with **both** assays showing NanoFlu titers appearing **less** than Fluzone HD

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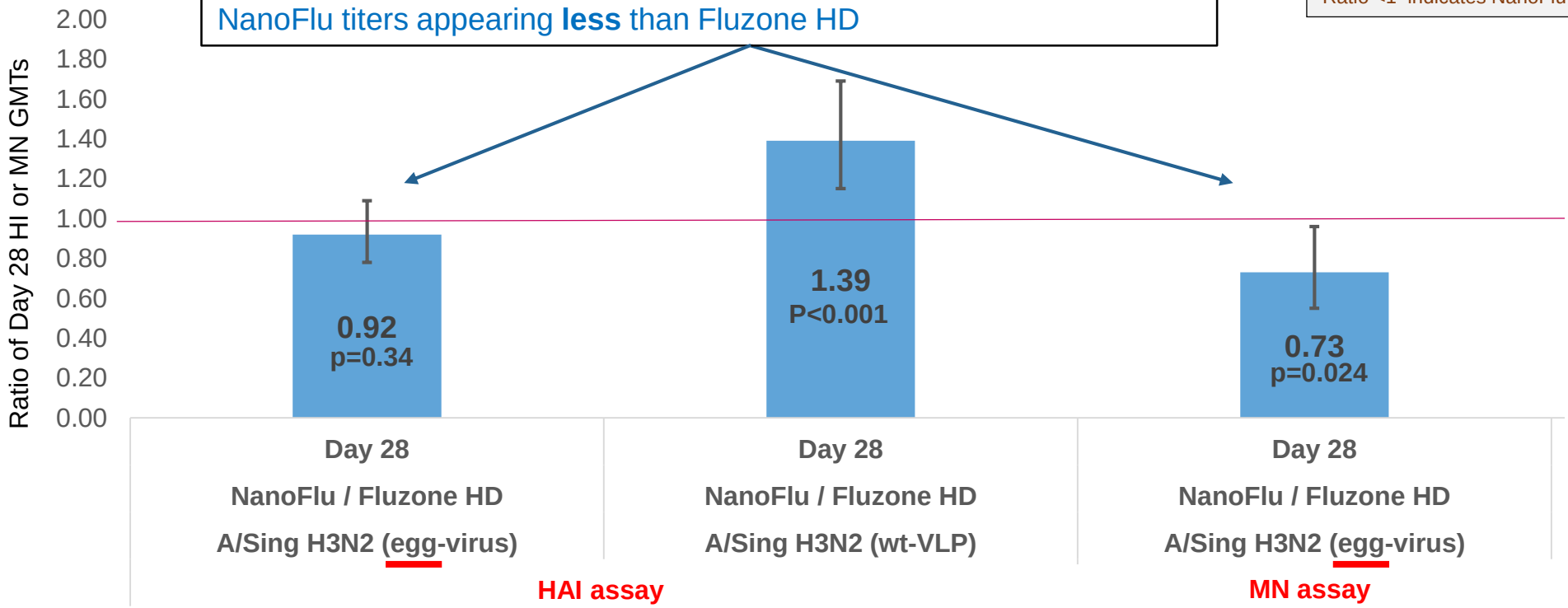

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Use of ***wild-type virus*** **reagents on MN** recapitulated results of ***wild-type VLP*** **reagents on wt-HAI,** with both assays showing NanoFlu titers appearing significantly **greater** than Fluzone HD titers

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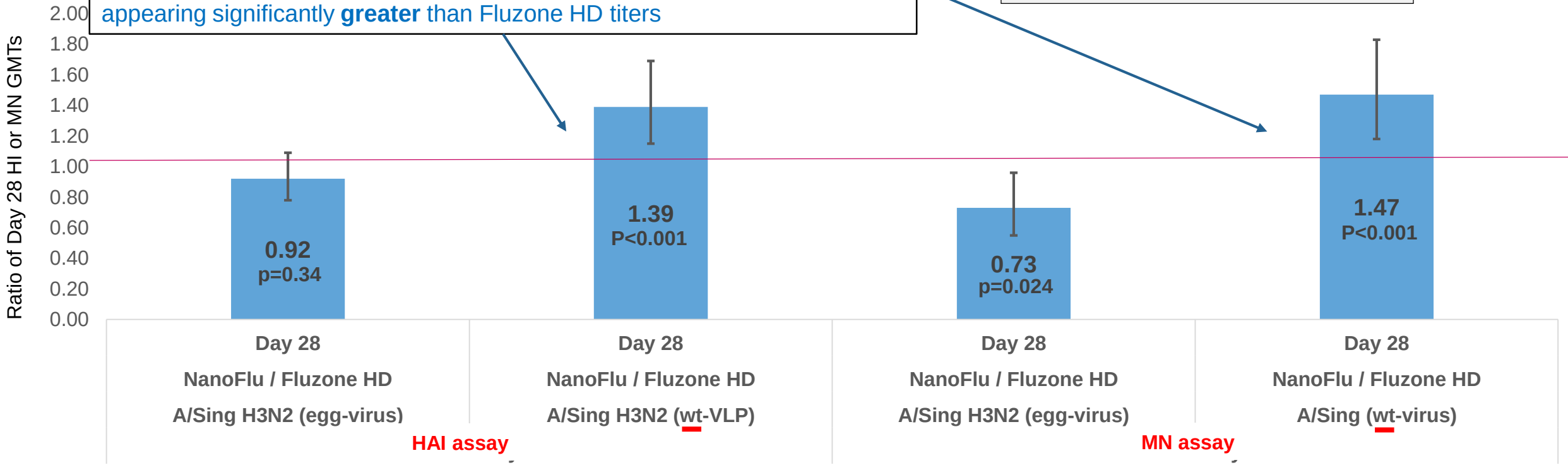

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MN antibody results confirmed the validity of the wt-VLP HAI system, by recapitulating differences seen with egg-virus vs wt-VLP HAI reagents

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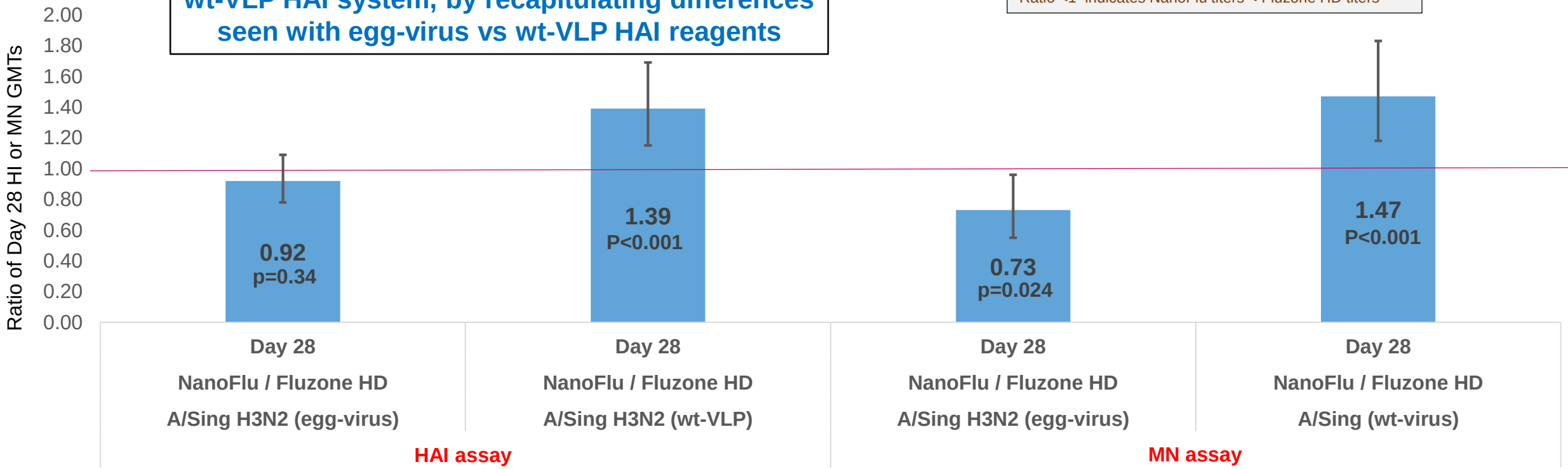

Summary

- Historically, c-HAI has been a useful serological tool
- Several critical problems of the current c-HAI format currently limit its utility
  - Failure to agglutinate A/H3N2 viruses, and therefore, measure HAI responses to A/H3N2 viruses
  - Egg-adaptation bias in reagents limits c-HAI assay's clinical relevance
  - Creation of unintended barriers to entry for improved next-generation influenza vaccines
- We developed wt-HAI to address these problems with several assay modifications:
  - Use of VLPs expressing wild-type HAs; and use of human type-O RBCs
- We assessed wt-HAI assay performance and established:
  - The improved agglutinating potential of wild-type VLPs + human type-O RBCs for A/H3N2 viruses
  - The comparability of HAI responses of wt-HAI and c-HAI to historical reference antisera
  - The confirmation of validity of wt-HAI using the wt-MN assay

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Thank you

Backups

Assessment of the agglutinating potential of combinations of RBCs and cell-passaged virus or VLPs:
A combination of VLPs and human RBCs yielded significantly better hemagglutination titers than either alone

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A/Switzerland/97/15293/13 (H3)	VLP	**2,048**	**2,048**	**8,192**
A/Switzerland/97/15293/13 (H3)	MDCK virus	256	64	128
A/Texas/50/12 (H3)	VLP	**8,192**	**8,192**	**32,768**
A/Texas/50/12 (H3)	MDCK virus	16	16	128
A/Michigan/45/15 (H1)	VLP	**16,384**	**8,192**	**32,768**
A/Michigan/45/15 (H1)	MDCK virus	64	32	128
B/Brisbane/60/08 (Victoria)	VLP	**16,384**	**8,192**	**32,768**
B/Brisbane/60/08 (Victoria)	MDCK virus	64	64	512
B/Phuket/3073/13 (Yamagata)	VLP	**32,768**	**16,384**	**65,536**
B/Phuket/3073/13 (Yamagata)	MDCK virus	64	128	1,024
B/Colorado/06/17 (Victoria)	VLP	262,144	131,072	65,536
B/Colorado/06/17 (Victoria)	MDCK virus	256	256	1,024