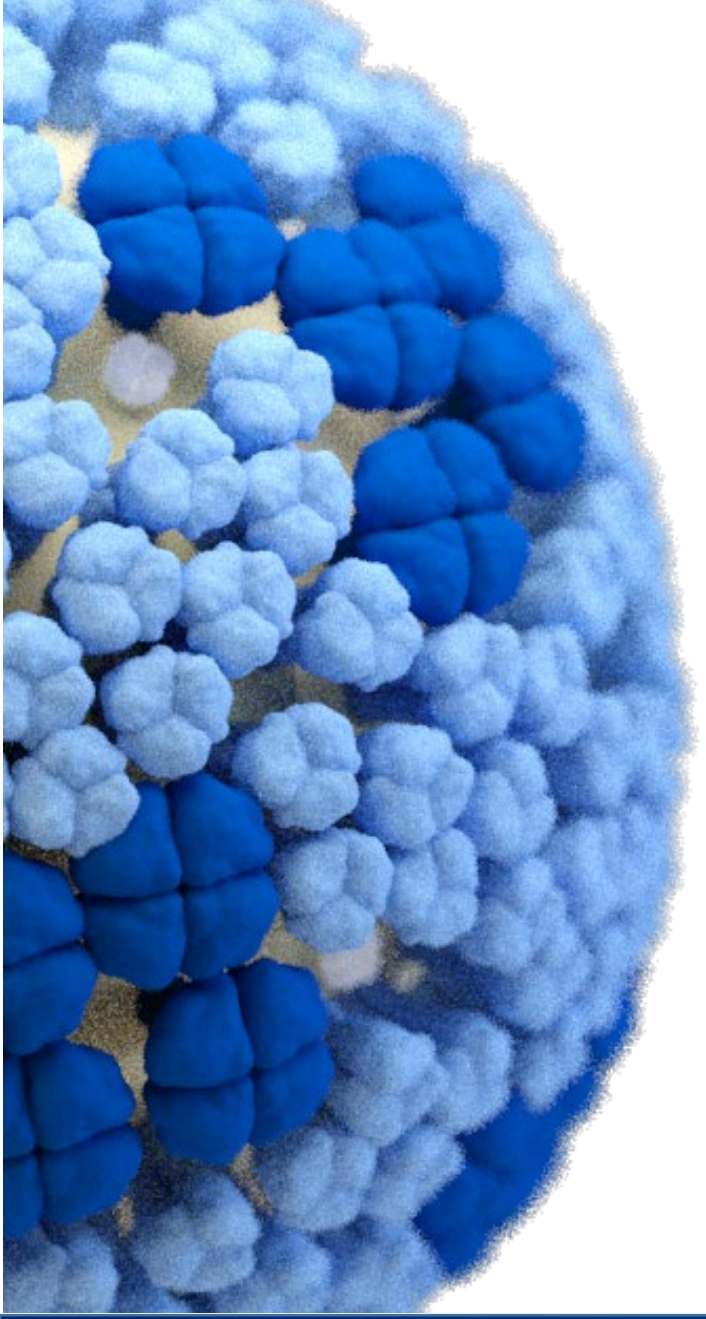

Human Influenza Vaccine Antigen Selection

David E. Wentworth, Ph.D.
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Chief, Virology Surveillance and Diagnosis Branch
Influenza Division, National Center for Immunization and Respiratory Diseases
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Outline

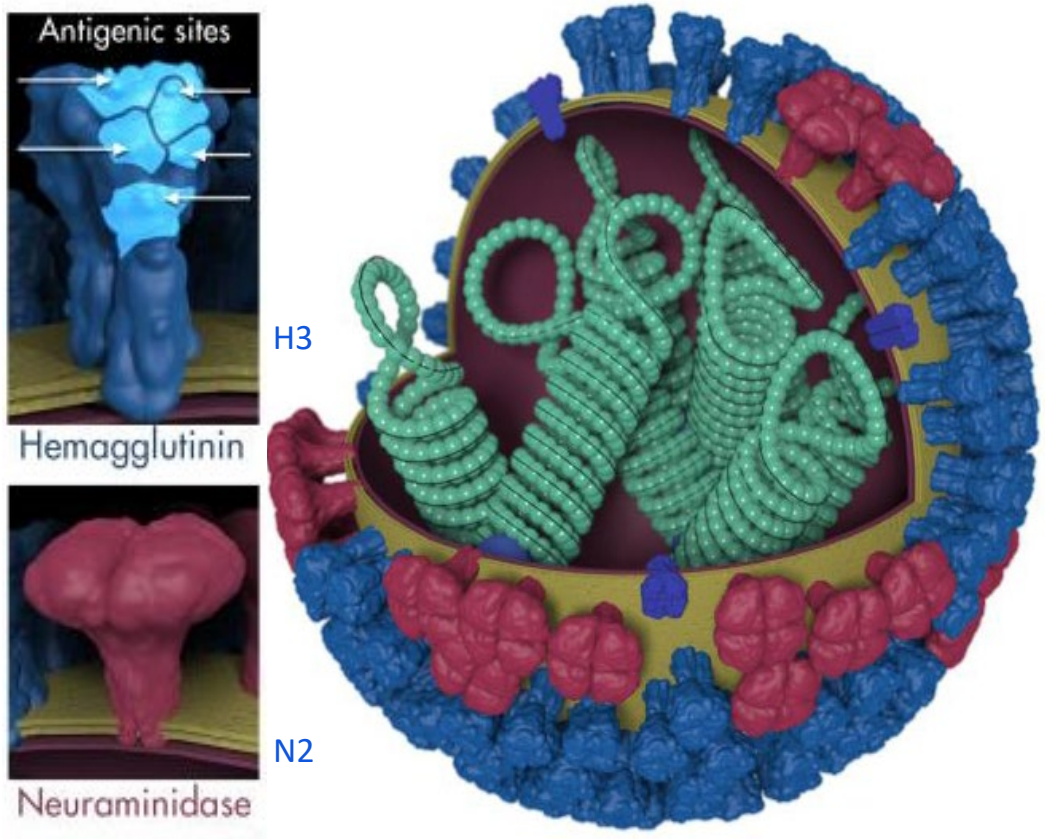
Background on the viruses we are fighting and the inherent challenges they pose to developing vaccines

What are the key questions addressed for vaccine antigen composition and the types of data needed

Examples of how these questions are addressed

Influenza A and B Viruses

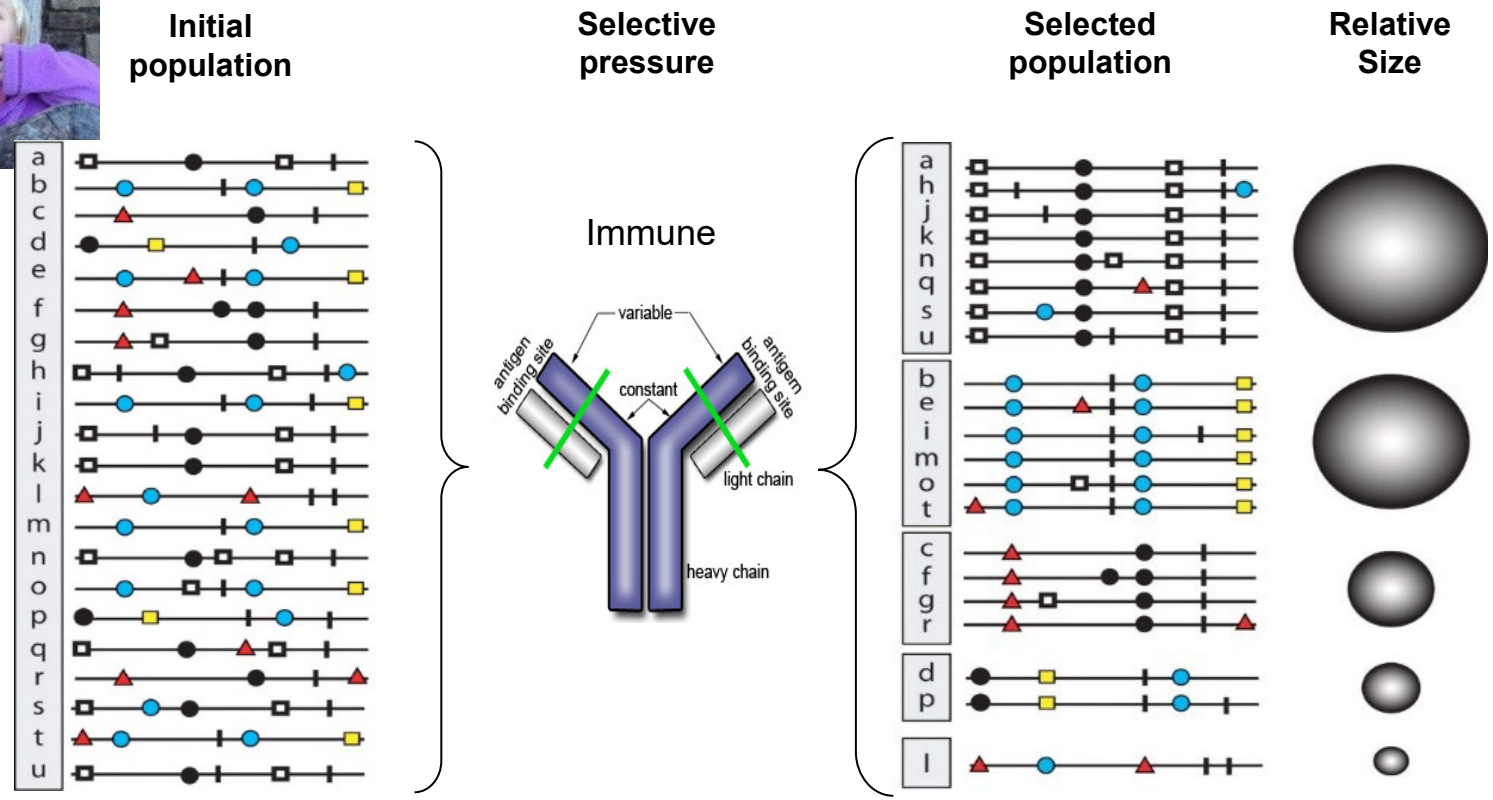
- Influenza A/B viruses of humans
  - Influenza A(H3N2)
  - Influenza A(H1N1)pdm09
  - Influenza B/Victoria
  - Influenza B/Yamagata
    - Not detected since March 2020
- Important surface proteins
  - Hemagglutinin – Vaccines induce antibodies to block its function
  - Neuraminidase – Antibodies and antiviral drugs inhibit this protein
- Genome: 8 segments negative sense RNA
  - Enables reassortment and high error rate



Influenza Viruses Survive On The Edge of Catastrophe

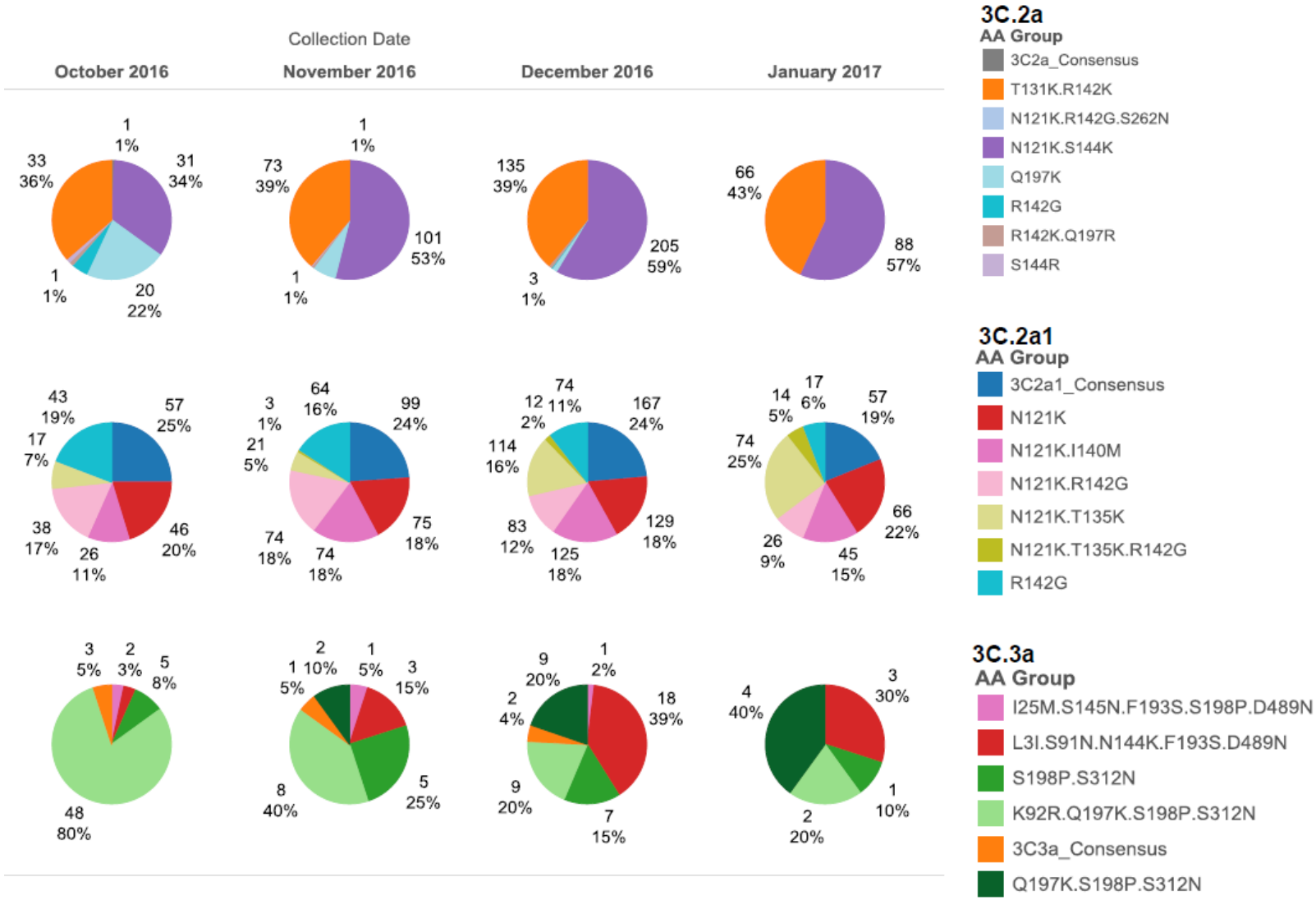
- Influenza viruses are constantly changing
  - Requires continuous comprehensive virus surveillance
  - Necessitates frequent updates to the vaccine
- Replication of influenza viruses is error-prone
  - Disadvantage for the virus
    - Close to the threshold of extinction (e.g., many defective viruses)
  - Advantages for the virus
    - Increased adaptability, variants are rapidly selected upon any type of evolutionary pressure (e.g., antiviral drugs, new host, immune)
    - Evolutionary benefit for evading host immunity
- Influenza survives as a population of viruses, not as a single virus

Why Existing as Population of Variants is Important



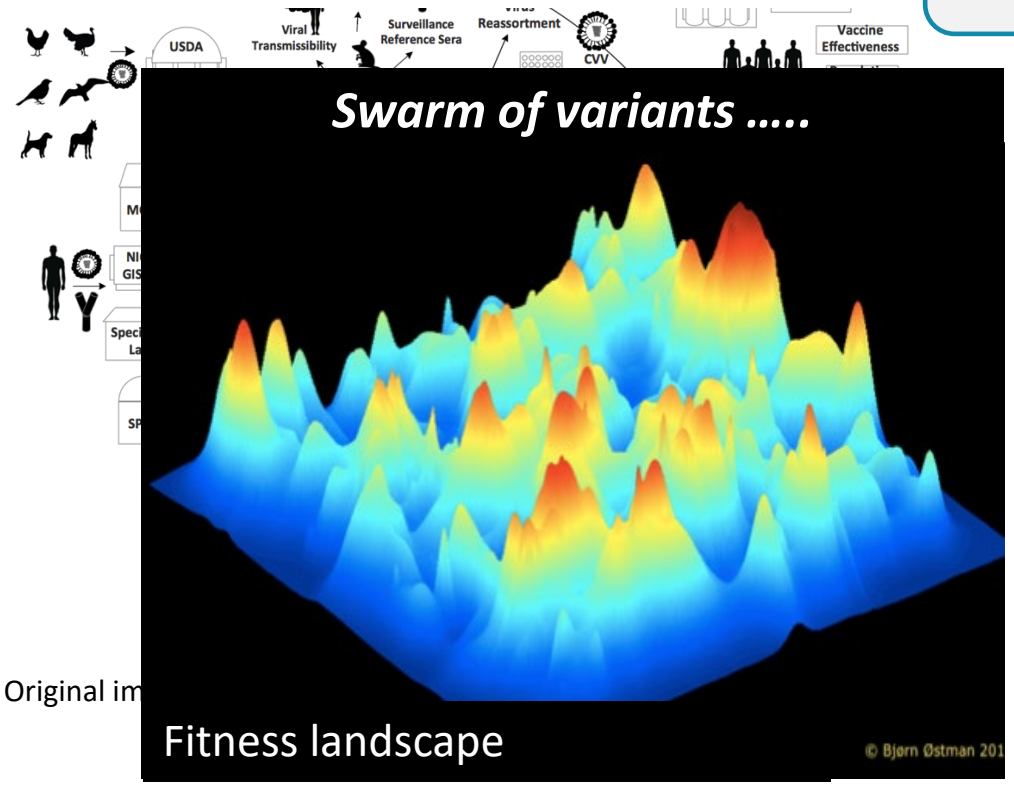
Modified from Domingo E et al. Microbiol. Mol. Biol. Rev. 2012;76:159-216

Example of Co-circulation Dynamics H3 HA Gene Variants



Selecting Four Vaccine Antigens Six Months in Advance is Complicated

Analyzing Complex Biological System



Original im

What's that, you need an accurate prediction 6 months from now and how to stop it! ☹

Key Questions Addressed As Part of Influenza Virus Vaccine Antigen Recommendations

Goal is to identify antigen(s) that elicit immunity to protect against diverging viruses that will likely co-circulate in the future

- Are/were there significant epidemics and where were they?
- What are the genetic subclades (variants) that have emerged in our population?
- Are the new emerging variants spreading geographically?
- Are emerging variant viruses antigenically distinct from prior or contemporary viruses?
- What is the proportion of the new group(s) and what group(s) is/are likely to predominate?
- Do current vaccines induce antibodies in humans that protect against co-circulating viruses and/or emerging variants?
  - For each of the 4 groups (type/subtype/lineage)?
- Is the current vaccine antigen likely to provide the best protection, or is a new prototype needed?

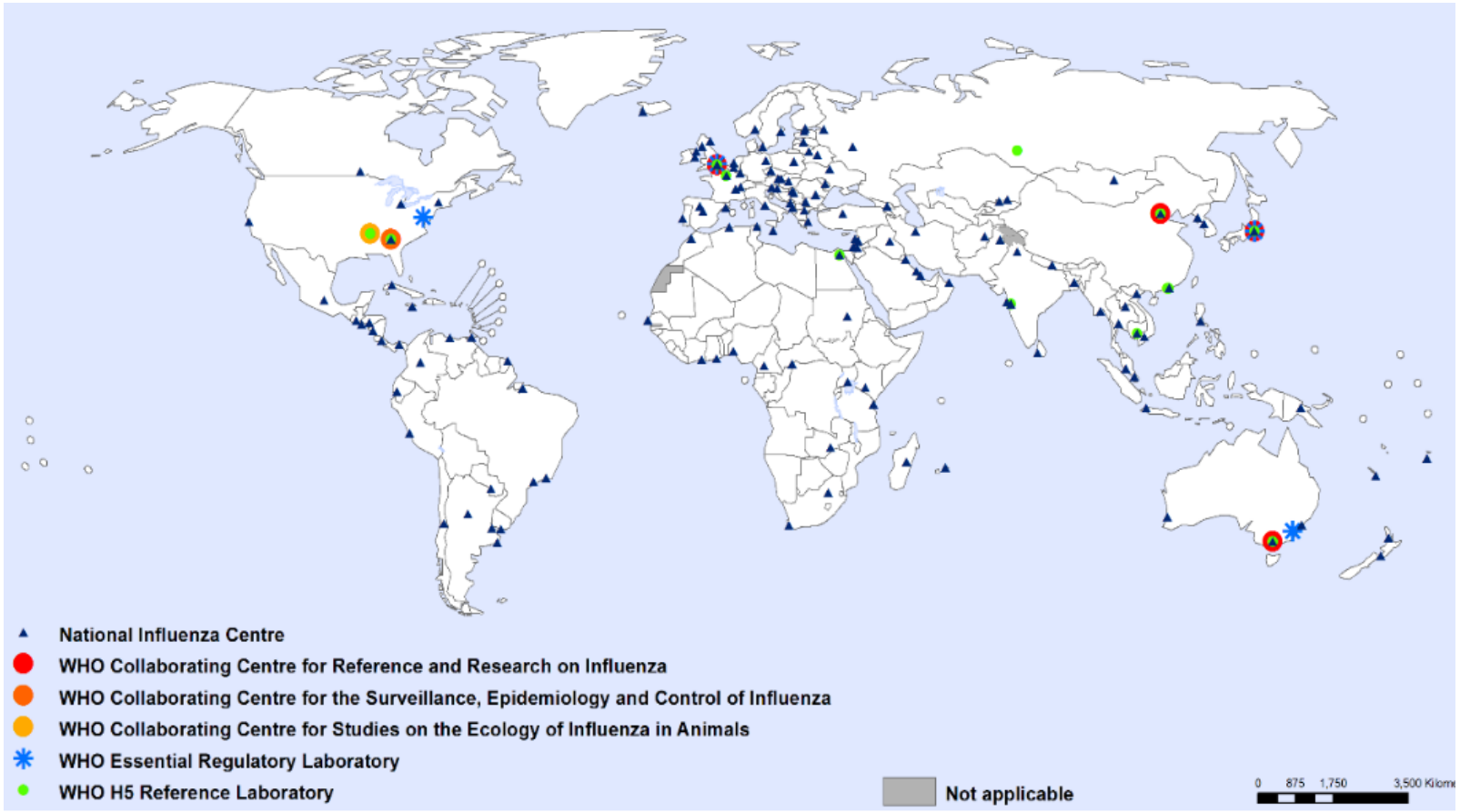
Data Used to Address Key Questions

- **Epidemiologic and clinical data**
  - Where are recent epidemics occurring, are they unusual in magnitude or disease ?
- **Virus surveillance** (*GISRS: 70 years in the making*)
  - GISRS labs test 50-150 thousand samples per week year-round and identify influenza positive specimens
    - Four virus groups: A(H1N1)pdm09, A(H3N2), B/Victoria, B/Yamagata, enabled by training, diagnostic kits (e.g., Dx rtRT-PCR , EQAP)
  - Regularly share representative specimens to WHO-CCs
- **Genomic characterization of viruses** (*Influenza changes rapidly and multiple subclades of interest continually emerge*)
  - Primary focus are HA and NA genes, conduct genome constellation analysis and identify reassortants, patterns of parallel/convergent evolution
- **Antigenic characterization of representative emerging viruses**
  - Level of antigenic drift from progenitors and/or vaccine references
    - Naïve animal models used to determine level of antigenic variation ("drift)understand immune response triggered by the proteins on the surface of influenza virus to determine if they would be neutralized by the current vaccine, or have the potential to be a new vaccine virus,
  - Emerging antigenically distinct variants are selected early as new reference viruses for serological analysis and as candidate vaccines (two-way characterization)
- **Data integration and comparison among WHO-CCs** (*shared data methods, reagents, and viruses*)
  - Influenza epidemiology, surveillance, phylogenetics, phylogeography, and antigenic data integration
  - Antigenic chartography, fitness forecasting
- **Post vaccination human serology studies**
  - Comparative analysis of cocirculating antigenic variants to identify those that pose the greatest risk of immune escape
- **Vaccine effectiveness studies** (*global consortium*)
  - VE lower than expected, decreasing and/or show clade/subclade specific VE differences identified *(data on the previous selections and their continued utility)*
- **Availability and characteristics of new candidate vaccine virus antigens**
  - Data generated that illustrates the new antigens induce antibodies that neutralize viruses most likely to co-circulate in upcoming seasons or are cross-protective (progenitors and/or emerging variants)

Global Influenza Surveillance and Response System (GISRS) Critical to Vaccine Antigen Updates

Conducting continuous surveillance

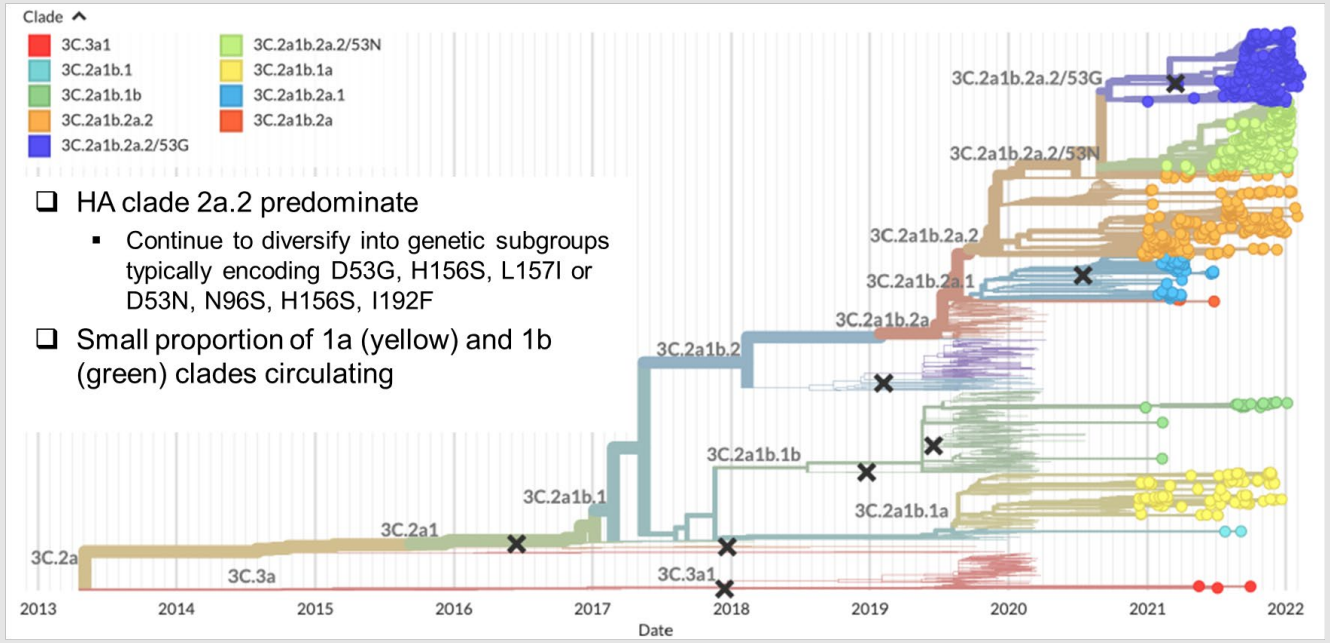
- 147 WHO National Influenza Centers in 123 Member States
  - 50,000-150,000/wk year-round
  - Share representative specimens with WHO-CCs
- 7 WHO Collaborating Centers for Influenza
- 4 Essential Reference Laboratories
- 12 WHO H5 Reference Laboratories



https://www.who.int/initiatives/global-influenza-surveillance-and-response-system

Genotype to phenotype analysis now central to vaccine antigen selection

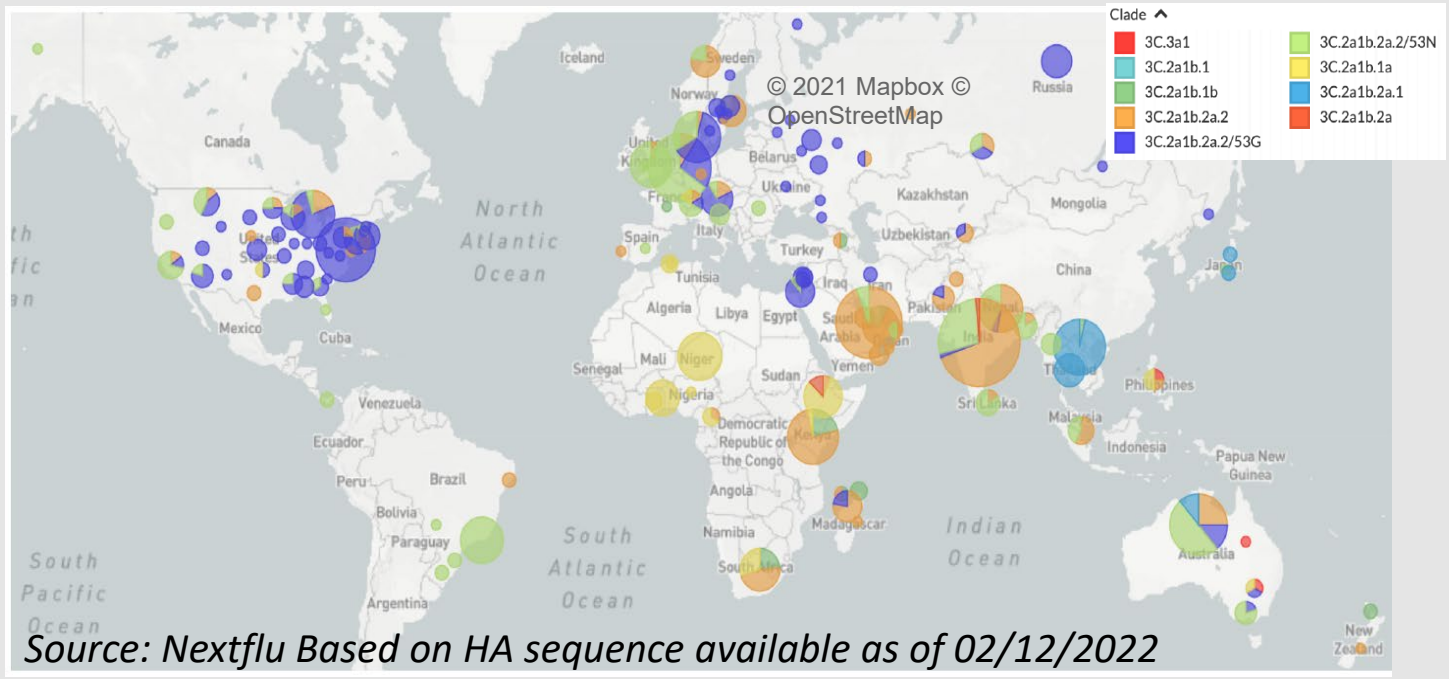
Phylogenetics of A(H3N2) HA Gene (time tree)



Source: Nextflu (J. Huddleston, T. Bedford, J. Lee & R. Neher). Based on HA sequences available as of 02/12/2022

- **Identify emerging lineages early**
  - **National influenza centers (NICs)**
  - **Regularly share samples to WHO-CCs and they are sequenced and deposited into databases**
  - **Some NICs sequence and publish in databases**
- Are they disseminating (country/global)
- Are they antigenically distinct
- To what level do they escape antibodies elicited by vaccine antigen?
- Data integration and fitness forecasting

Genotype to phenotype analysis now central to vaccine antigen selection



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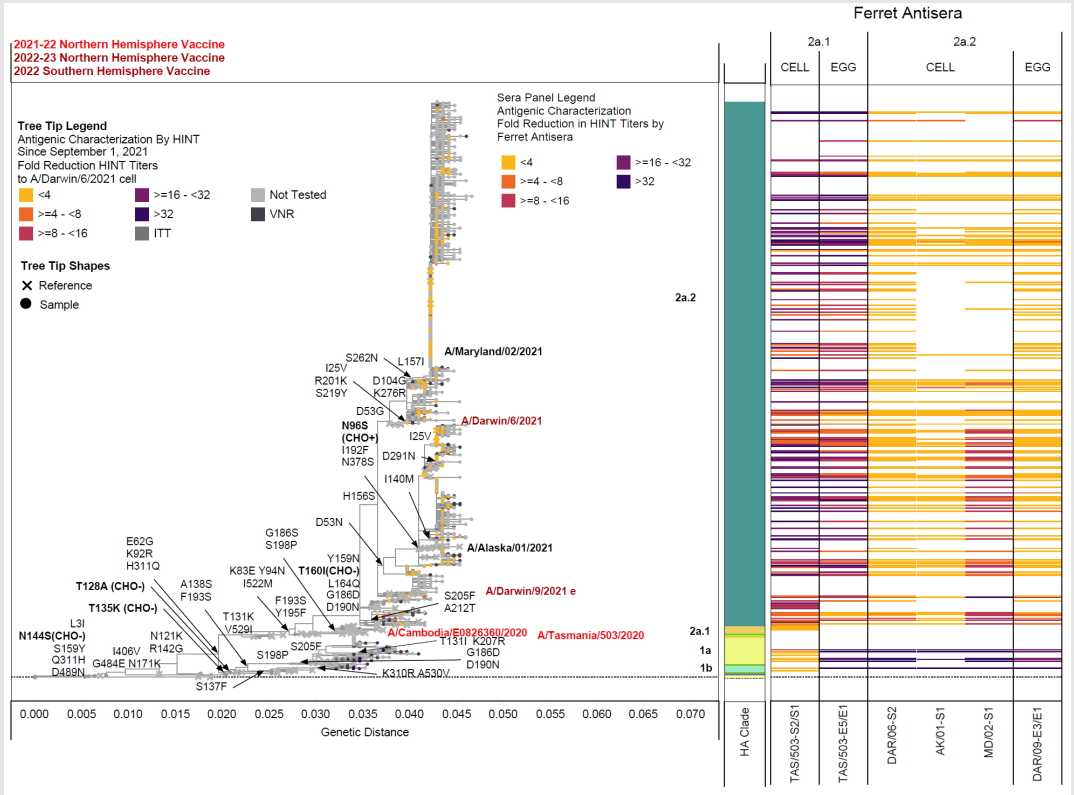

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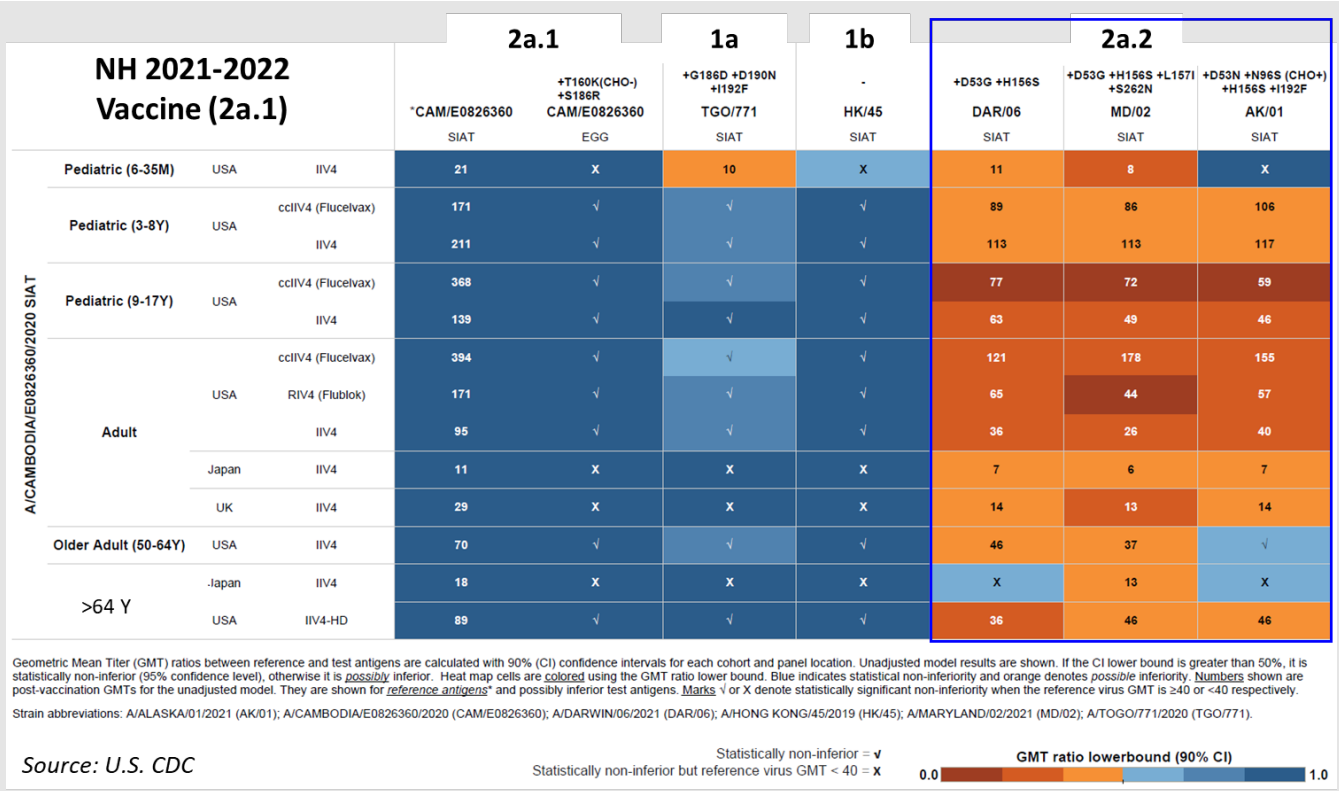

NH 2021-2022 Vaccine (2a.1)			2a.1		1a	1b	2a.2		
			*CAM/E0826360 SIAT	+T160K(CHO-) +S186R CAM/E0826360 EGG	+G186D +D190N +I192F TGO/771 SIAT	- HK/45 SIAT	+D53G +H156S DAR/06 SIAT	+D53G +H156S +L157I +S262N MD/02 SIAT	+D53N +N96S (CHO+) +H156S +I192F AK/01 SIAT
A/CAMBODIA/E0826360/2020 SIAT	Pediatric (6-35M)	USA	IIV4	21	X	10	X	11	8	X
	Pediatric (3-8Y)	USA	ccIIV4 (Flucelvax)	171	√	√	√	89	86	106
			IIV4	211	√	√	√	113	113	117
	Pediatric (9-17Y)	USA	ccIIV4 (Flucelvax)	368	√	√	√	77	72	59
			IIV4	139	√	√	√	63	49	46
	Adult	USA	ccIIV4 (Flucelvax)	394	√	√	√	121	178	155
			RIV4 (Flublok)	171	√	√	√	65	44	57
			IIV4	95	√	√	√	36	26	40
		Japan	IIV4	11	X	X	X	7	6	7
		UK	IIV4	29	X	X	X	14	13	14
	Older Adult (50-64Y)	USA	IIV4	70	√	√	√	46	37	√
	>64 Y	Japan	IIV4	18	X	X	X	X	13	X
		USA	IIV4-HD	89	√	√	√	36	46	46

Geometric Mean Titer (GMT) ratios between reference and test antigens are calculated with 90% (CI) confidence intervals for each cohort and panel location. Unadjusted model results are shown. If the CI lower bound is greater than 50%, it is statistically non-inferior (95% confidence level), otherwise it is *possibly* inferior. Heat map cells are colored using the GMT ratio lower bound. Blue indicates statistical non-inferiority and orange denotes *possible* inferiority. Numbers shown are post-vaccination GMTs for the unadjusted model. They are shown for *reference antigens** and possibly inferior test antigens. Marks √ or X denote statistically significant non-inferiority when the reference virus GMT is ≥40 or <40 respectively.

Strain abbreviations: A/ALASKA/01/2021 (AK/01); A/CAMBODIA/E0826360/2020 (CAM/E0826360); A/DARWIN/06/2021 (DAR/06); A/HONG KONG/45/2019 (HK/45); A/MARYLAND/02/2021 (MD/02); A/TOGO/771/2020 (TGO/771).

Source: U.S. CDC

Statistically non-inferior = √
Statistically non-inferior but reference virus GMT < 40 = X

GMT ratio lowerbound (90% CI)
0.0 — 1.0

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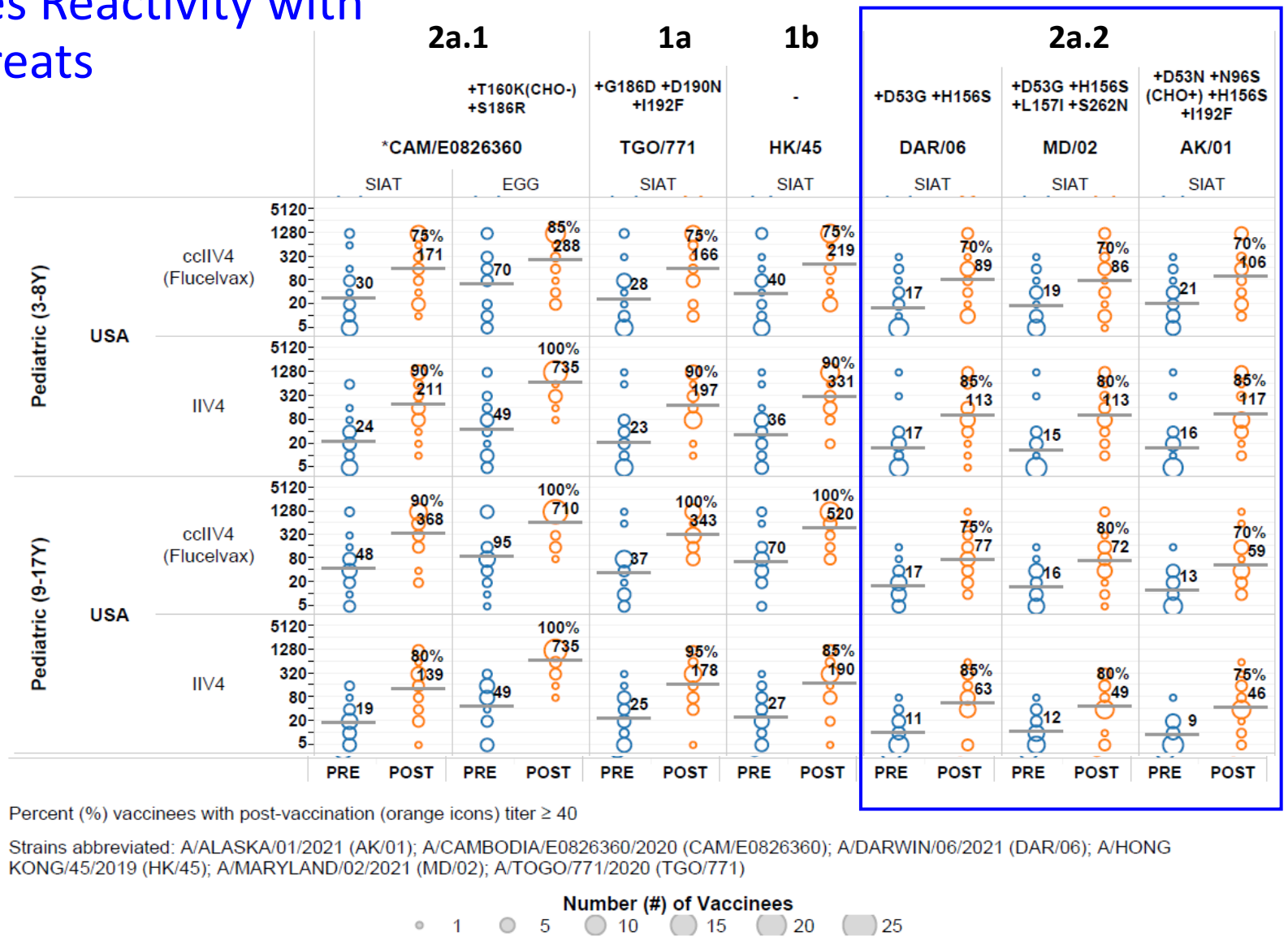
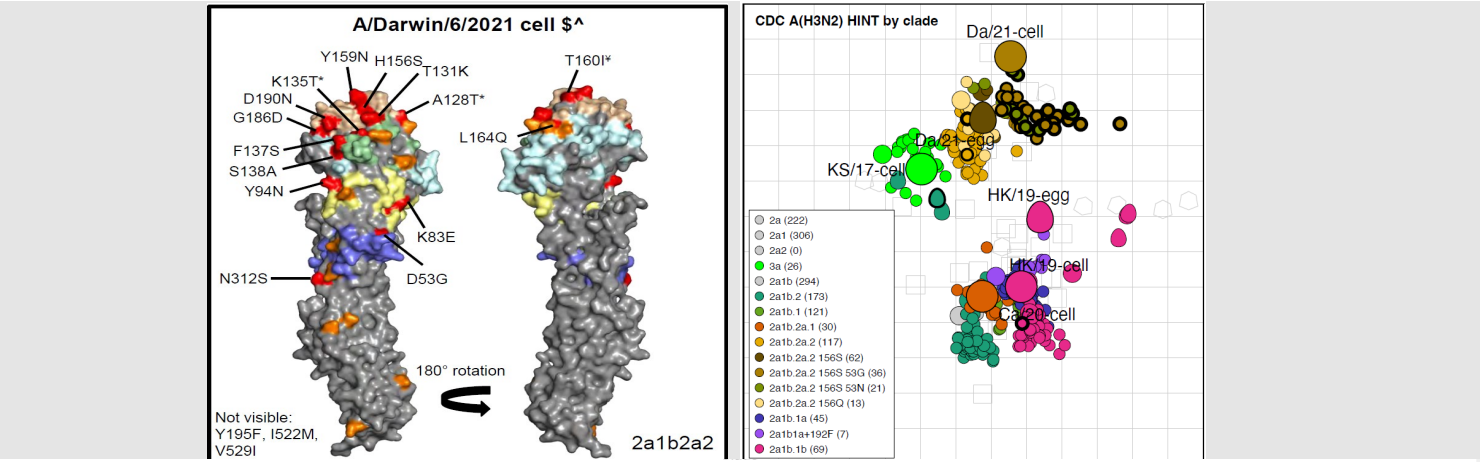
Human Post-vaccination Sera Analysis of A(H3N2) Viruses Illustrates Reactivity with Emerging Threats

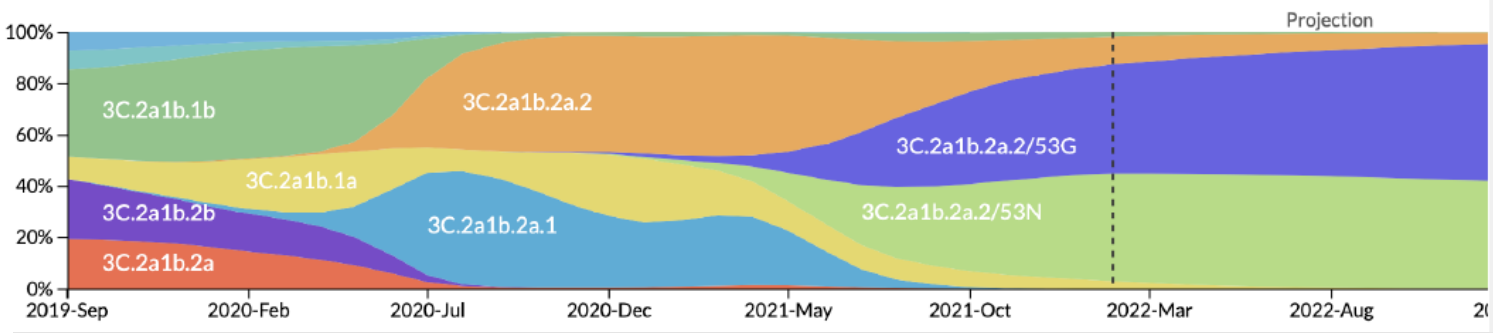
NH 2021-2022 Vaccine (2a.1), Individual Responses (Older pediatric)

- Vaccination increased titers to HA clade 1a, 1b, 2a.1 and 2a.2 viruses
- Boost immune memory (HK/45 (1b) (*back*)
- Boost immunity to emerging viruses (*forward*)
  - Emerging 1a (TGO/771)
  - Multiple emerging 2a.2 variants
    - DAR/06 (D53G, H156S)
    - MD/02 (D53G, H156S, L157I)
    - AK/01 (D53N...I192F)



Genotype to phenotype analysis now central to vaccine antigen selection





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- **Data integration and fitness forecasting**
  - **How fit is new lineage among other circulating and emerging lineages**
  - **Identify optimal vaccine antigen**
    - **Which virus antigens induce protection from emerging group(s) and other co-circulating virus groups**

Analysis of Candidate Vaccine Viruses

Ferret antisera to:

- SH 2021 reference viruses
  - Inhibit clade 1a and 1b
  - Poorly inhibited clade 2a1 and 2a2 viruses
- NH 2021-22 reference viruses
  - Inhibited 1a, 1b and 2a1 viruses
  - Reduced inhibition of clade 2a2 viruses
- 2a2 reference viruses
  - Well inhibited 2a2
  - Reduced inhibition of 2a1
  - Poor inhibition of 1a and 1b viruses

SH 21 | NH 21-22 | Potential SH 22

			Refference Ferret Antisera									< 4 fold
												4 fold
			Dar/ 726	HK/ 2671	Cam/ e0826360	Cam/ e0826360	Bang/ 10006	Dar/ 6	Dar/ 11	Dar/ 9		8 fold
			CELL	EGG	CELL	EGG	CELL	CELL	QMC2	EGG		>8 fold
Reference Antigens	**Passage**	**Clade**	**3C.2a1b. 1b**	**3C.2a1b. 1b**	**3C.2a1b. 2a1**	**3C.2a1b. 2a1**	**3C.2a1b. 2a2**	**3C.2a1b. 2a2**	**3C.2a1b. 2a2**	**3C.2a1b. 2a2**		**Collection Date**
A/Perth/20/2020	MDCK-1, SIAT2	3C.2a1b. 1a	320	80	320	80	160	<40	80	80		
A/Darwin/726/2019	SIAT2	3C.2a1b. 1b	**640**	40	160	<40	<40	<40	<40	<40		
A/Hong Kong/2671/2019	E9	3C.2a1b. 1b	1280	**640**	80	160	160	80	80	160		
A/Cambodia/e0826360/2020	SIAT2	3C.2a1b. 2a1	40	<40	**320**	40	80	<40	40	80		
A/Cambodia/e0826360/2020	E5	3C.2a1b. 2a1	40	40	160	**320**	320	160	40	160		
A/Bangladesh/10006/2020	S3, SIAT1	3C.2a1b. 2a2	80	40	320	160	**320**	320	160	320		
A/Darwin/6/2021	SIAT2	3C.2a1b. 2a2	<40	<40	40	80	160	**640**	160	160		
A/Darwin/11/2021	QMC2	3C.2a1b. 2a2	40	<40	160	80	160	640	**160**	160		
A/Darwin/9/2021	E4	3C.2a1b. 2a2	40	<40	160	160	640	640	320	**320**		
Test Antigens												
A/Philippines/1/2021	MDCK2, SIAT1	3C.2a1b.1a	320	<40	160	<40	<40	<40	<40	<40		05/14/21
A/Philippines/8/2021	SIAT1	3C.2a1b.1a	320	<40	160	<40	<40	<40	<40	<40		07/13/21
A/Yamagata/1/2021	hCK2, SIAT1	3C.2a1b.2a1	40	<40	320	40	40	<40	40	80		02/09/21
A/Darwin/17/2021	SIAT1	3C.2a1b.2a2	<40	<40	40	80	160	640	160	160		08/11/21
A/Darwin/18/2021	SIAT1	3C.2a1b.2a2	<40	<40	40	80	160	1280	160	160		08/11/21
A/Darwin/19/2021	SIAT1	3C.2a1b.2a2	<40	<40	40	80	160	1280	160	160		08/11/21
A/Darwin/23/2021	SIAT1	3C.2a1b.2a2	<40	<40	<40	40	80	320	80	80		08/12/21
A/Darwin/24/2021	SIAT1	3C.2a1b.2a2	<40	<40	40	80	80	320	160	160		08/12/21
A/Nepal/NPWR-05637/2021	hCK2, SIAT1	3C.2a1b.2a2	80	40	160	160	320	320	160	320		04/08/21
A/Philippines/4/2021	MDCK2, SIAT1	3C.2a1b.2a2	<40	<40	160	80	160	640	160	160		06/24/21
A/Victoria/5/2021	SIAT2	3C.2a1b.2a2	<40	<40	40	80	80	320	160	160		08/11/21
A/Philippines/6/2021	MDCK2, SIAT1	3C.3a	<40	<40	40	<40	40	80	40	40		07/05/21

Source: VIDRL HI test

Summary

- Influenza viruses rapidly evolve and evade immunity generated from prior infection and/or vaccines
  - Many divergent populations are co-circulating
  - Evolve rapidly and escape host immunity
- Major goal is to identify antigen(s) that elicit immunity against diverging viruses that will likely co-circulate in the future
  - Use many data sources to address key questions related to emerging variants
    - Identification, dissemination, antigenic characterization, immune escape and risk
    - Genotype to phenotype approach and integration of that data now central vaccine antigen recommendations
- Many efforts are underway to overcome contemporary vaccine challenges and improve vaccine antigen selection
  - Increased use of Next-Generation sequencing
  - Data integration and fitness forecasting
  - Generation of many CVVs early and down select as vaccine recommendation date approaches

Acknowledgements I

- Global influenza surveillance and response system
  - National Influenza Centers (NICs)
  - WHO-Global Influenza Program and regional offices
  - Essential Regulatory Laboratories
  - WHO Collaborating Centers in Beijing, Melbourne, London and Tokyo and WHO Geneva staff
- US partners:
  - U.S. State Public Health Laboratories (64)
  - Association of Public Health Laboratories
  - United States Air Force School of Aerospace Medicine (USAFSAM)
  - Naval Health Research Center (NHRC)
- Data integration and fitness forecasting partners in Europe and US
  - D. Smith et. al., University of Cambridge partners
  - M. Lässig, M. Łuksza et. al.,
  - T. Bedford, R. Neher et. al.,
- CDC Influenza Division staff
  - Special thanks to Rebecca Kondor, Todd Davis, John Barnes, Larisa Gubareva, Min Levine, and John Steel

Acknowledgements II: CDC Influenza Division

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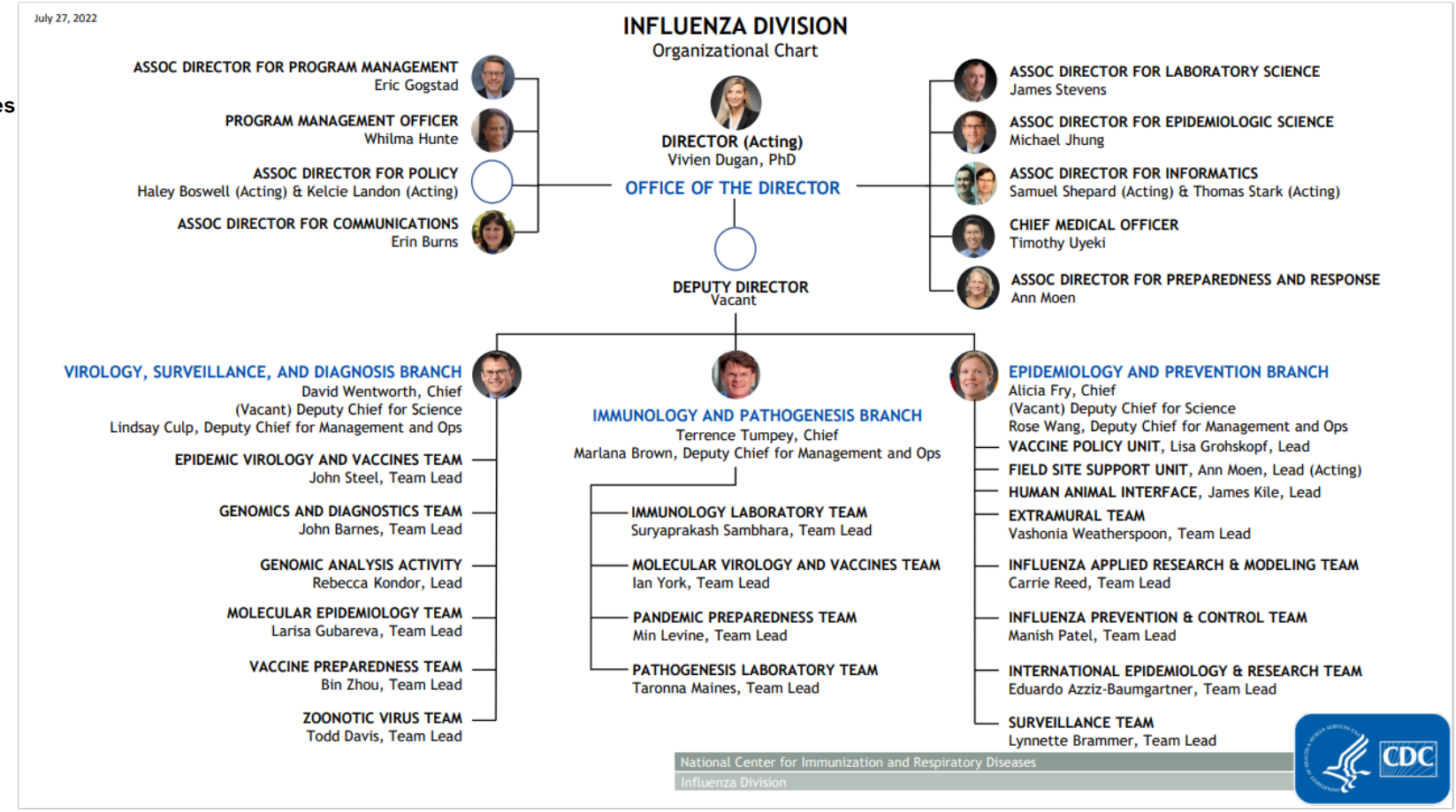

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