Influenza ("Flu") Virus is RNA Virus and has Types A & B & C:

Type C: not as much of health problem as Types A & B. Infects humans, dogs, and pigs.

Type B: Less common in humans than A (but Type B is not rare). Mutates and evolves more slowly than Type A. Infects humans, ferrets, and seals.

Type A:

1. The kind of flu that has been known to cause pandemics (and annual epidemics).
2. Infects humans, wild and domesticated birds, and (sometimes) pigs.
3. Evolves relatively quickly.
4. Divided into "serotypes" according to human antibody response to 2 proteins on surface of viral particles: Hemagglutinin (H) protein has 18 subtypes and Neuraminidase (N) has 11 subtypes. For example, H3N2 is influenza A with subtype #3 for hemagglutinin and subtype #2 for neuraminidase.

Influenza Type A details:

Genome has 11 genes encoded on 8 separate RNA pieces.

Not all infected people develop symptoms (maybe 1/3 do not?)

Infected people are typically highly infectious to others for about 5 days (starting about a day before they feel symptoms)

Infections can be spread through air, through mucus transferred from one person to another, and through bodily contact.

Overall, Influenza (Types A and B and C) together kill about 1/4 to 1/2 million people in typical years. High-risk groups are babies, the elderly, immunocompromised people, and pregnant women (see http://www.who.int/mediacentre/factsheets/fs211/en/)

1918 "Spanish" Flu Pandemic (summarized from wikipedia)

50-100 million people died (3-5% of world's population, possibly more deaths than can be connected to World War I)

unusual because it killed disproportionate number of young adults (rather than people with weak immune systems as flu usually does)

was the first H1N1 pandemic (may have been due to a virus originating in birds that was transferred to pigs and then to humans)

1968 "Hong Kong" Flu Pandemic - killed about a million people, was H3N2 (and descended from H2N2 via antigenic shift / reassortment)

2009 "H1N1" - came from a mix of North American avian flu, North American swine flu, and human influenza, and European or Asian swine flu

Young adults thought to have suffered much from 1918 flu due to "Cytokine Storms"

"... When the immune system is fighting pathogens, cytokines signal immune cells such as T-cells and macrophages to travel to the site of infection. In addition, cytokines activate those cells, stimulating them to produce more cytokines. Normally, the body keeps this feedback loop in check. However, in some instances, the reaction becomes uncontrolled, and too many immune cells are activated in a single place. ... If a cytokine storm occurs in the lungs, for example, fluids and immune cells such as macrophages may accumulate and eventually block off the airways, potentially resulting in death. ..."

above from wikipedia

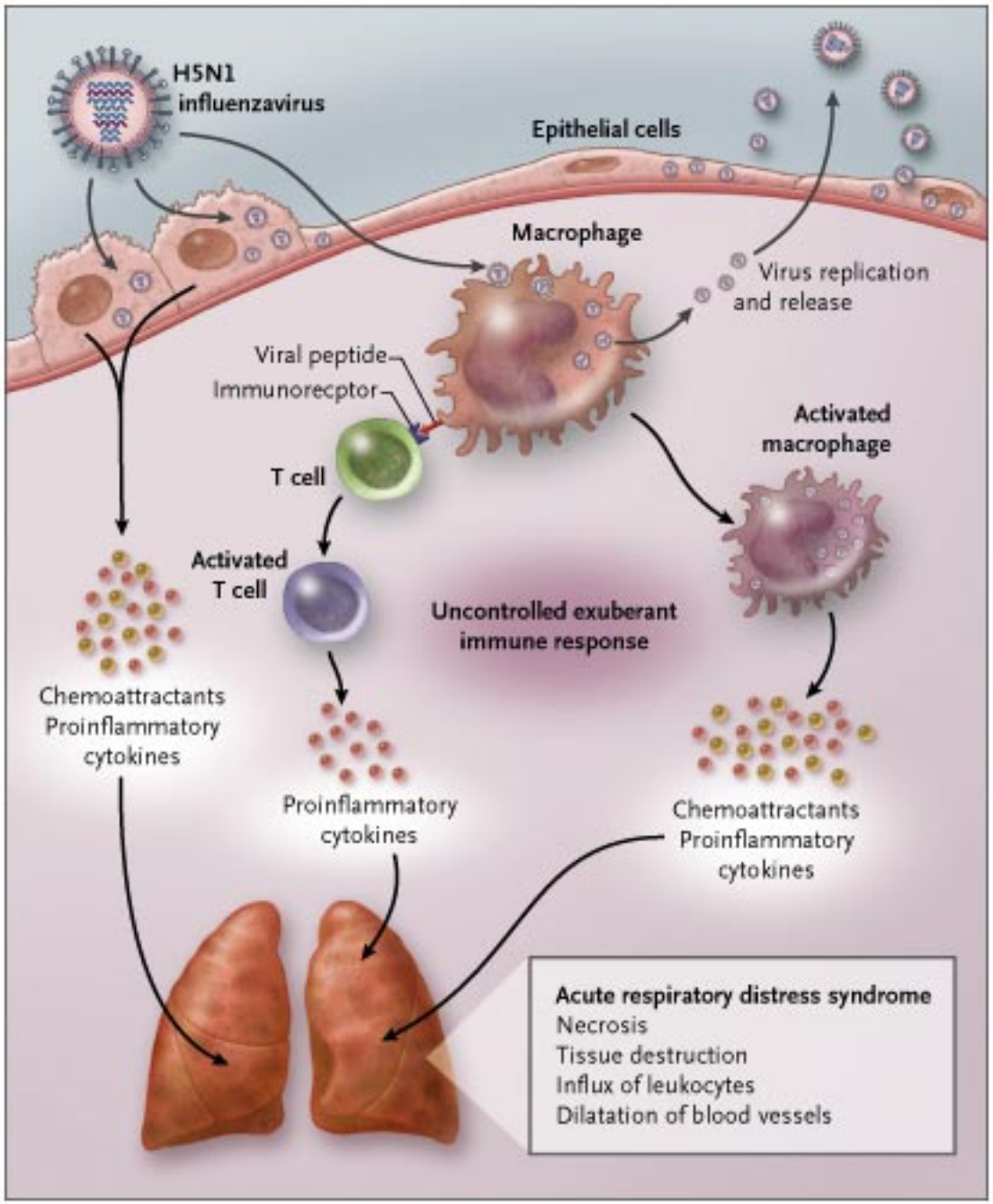


Figure from http://www.cytokinestorm.com

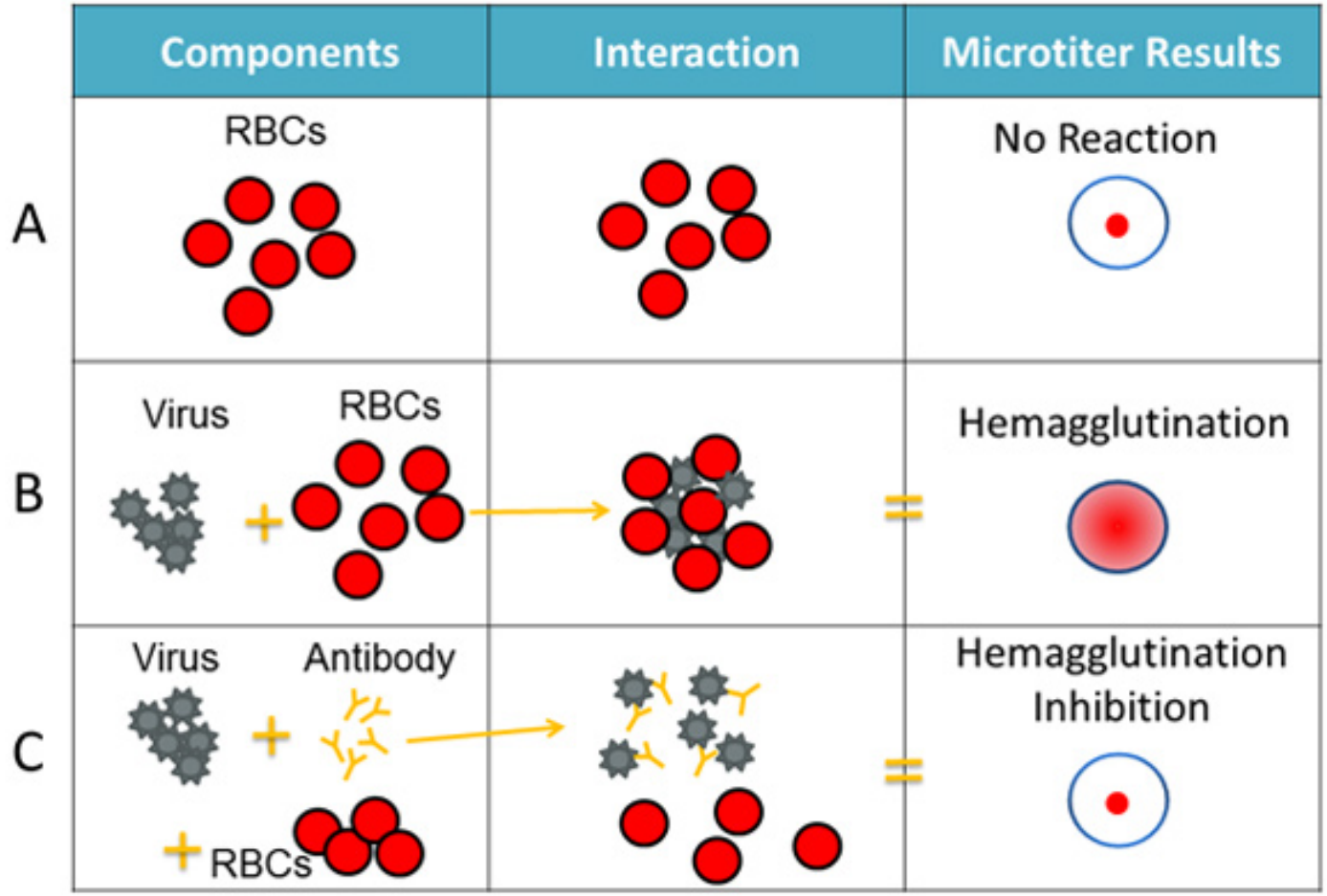
Antigenic Drift: Change of Antigens via viral mutation (typically a more gradual process)

Antigenic Shift: Change of Antigens via reassortment. Two or more virusses combine. Mix-and-match happens with the surface antigens. Can cause trouble for host because big "jump" in viral evolution can occur and host immune system may not have seen anything like it.

Antigenic shift may be facilitated by proximity between humans and domesticated pigs or fowl as well as by proximity between domesticated and wild fowl.

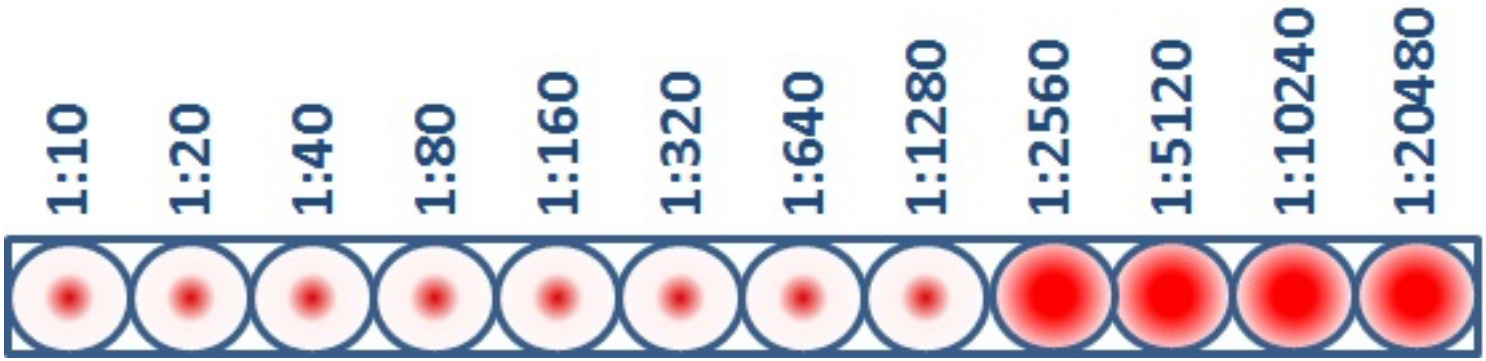
Hemagglutinin Inhibition Assay (HI Test)

from http://www.cdc.gov/flu/professionals/laboratory/antigenic.htm



"Row A shows that in the absence of virus, RBCs in a solution will sink to the bottom of a microtiter plate well and look like a red dot. Row B shows that influenza viruses will bind to red blood cells when placed in the same solution. This is called hemagglutination and is represented by the formation of the lattice structure, depicted in the far right column under "Microtiter Results." Row C shows how antibodies that are antigenically similar to a virus being tested will recognize and bind to that influenza virus. This prevents the virus and RBCs from binding, and therefore, hemagglutination does not occur ..."

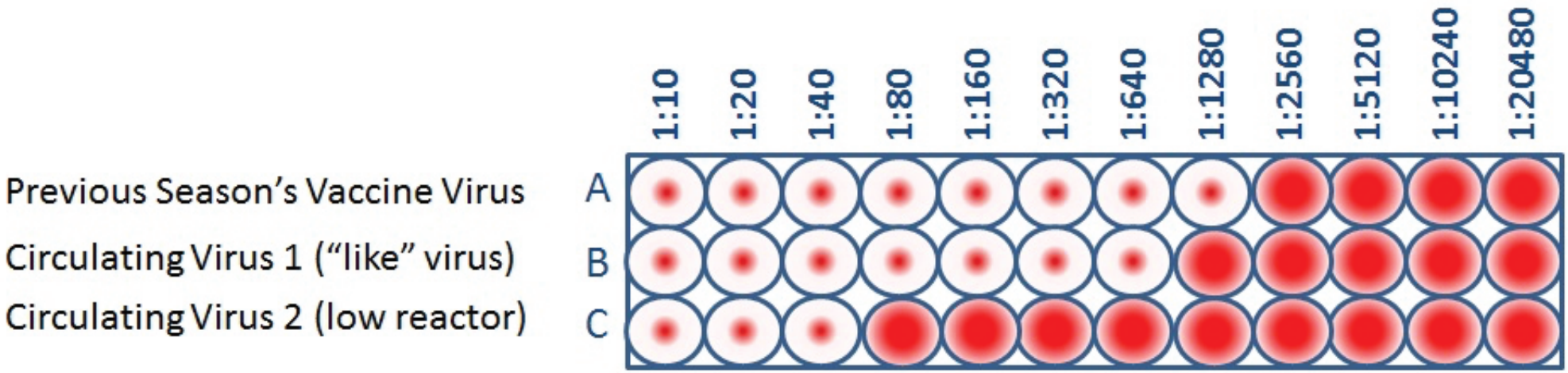
HI Titer Assay



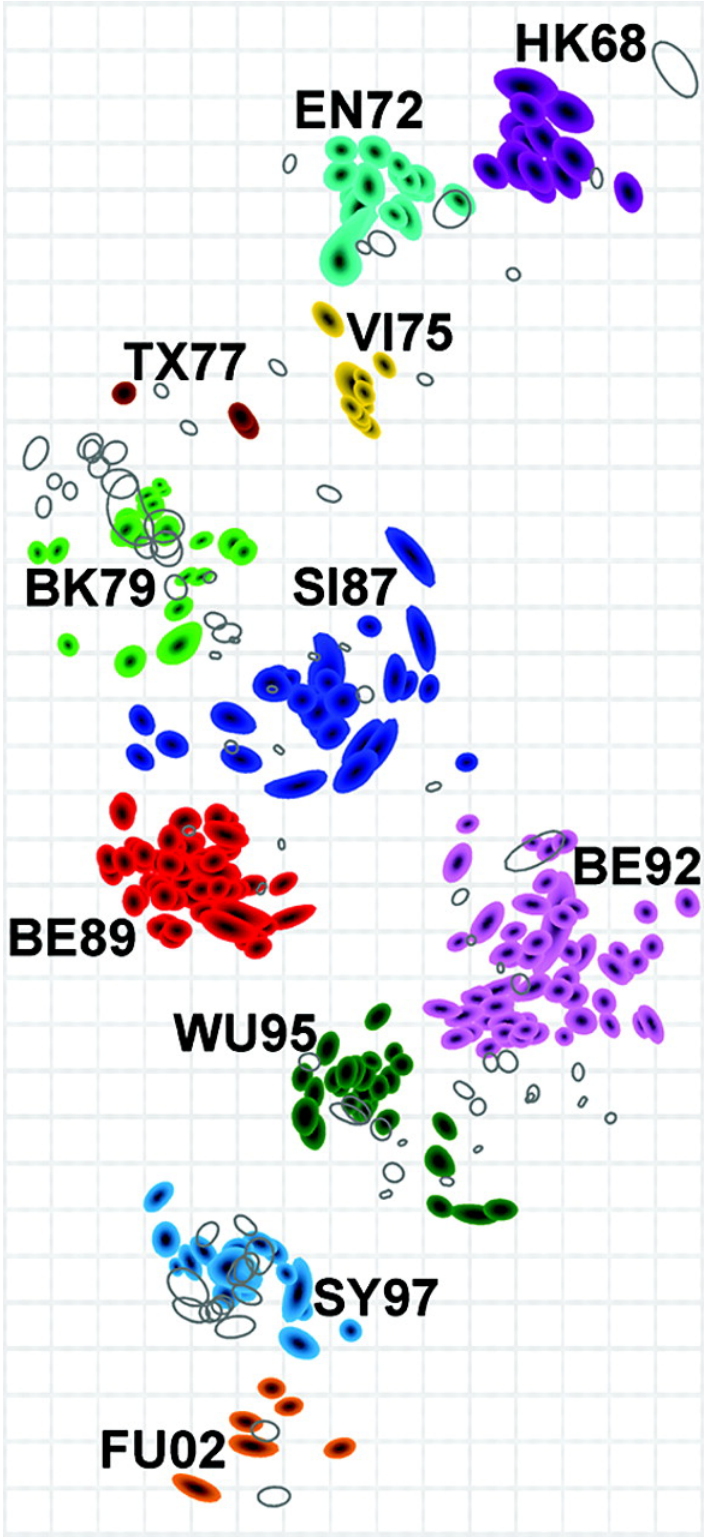
" This virus sample has an HI titer of 1280, which means that the greatest dilution of antibody that still blocked hemagglutination from occurring was at 1280 dilution. At this dilution, the antibodies were still capable of recognizing and binding to the antigens on the virus. ..."

from http://www.cdc.gov/flu/professionals/laboratory/antigenic.htm

Antigenic Characterization Assay



from http://www.cdc.gov/flu/professionals/laboratory/antigenic.htm



This is figure 1 from Smith et al. 2004. Science 305:371-376

"... Antigenic map of influenza A (H3N2) virus from 1968 to 2003. The relative positions of strains (colored shapes) and antisera (uncolored open shapes) were adjusted such that the distances between strains and antisera in the map represent the corresponding HI measurements with the least error ...
Strain color represents the antigenic cluster to which the strain belongs. Clusters were identified by a k-means clustering algorithm ... The vertical and horizontal axes both represent antigenic distance, and, because only the relative positions of antigens and antisera can be determined, the orientation of the map within these axes is free. The spacing between grid lines is 1 unit of antigenic distance— corresponding to a twofold dilution of antiserum in the HI assay. Two units correspond to fourfold dilution, three units to eight-fold dilution, and so on. ..."

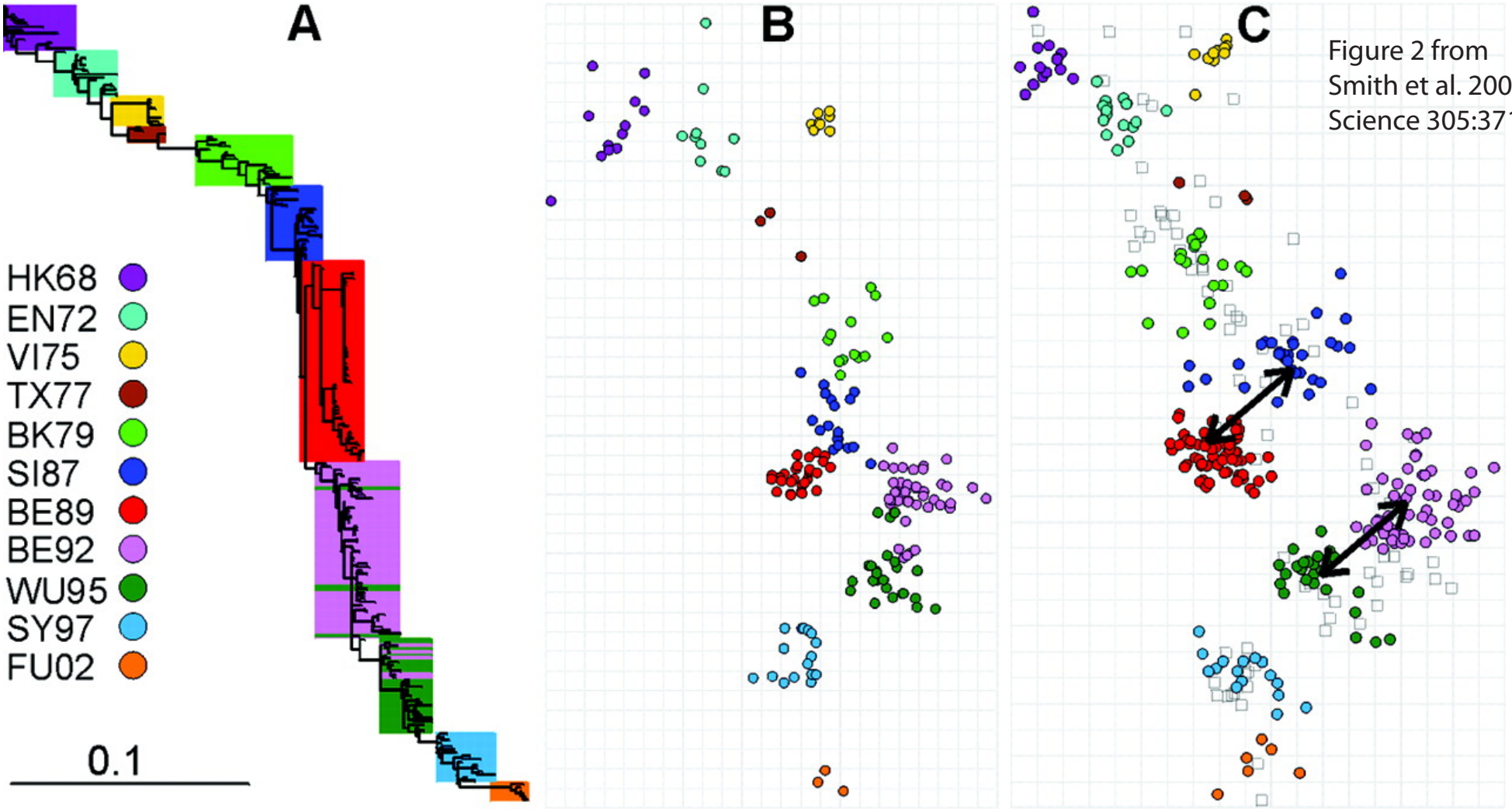
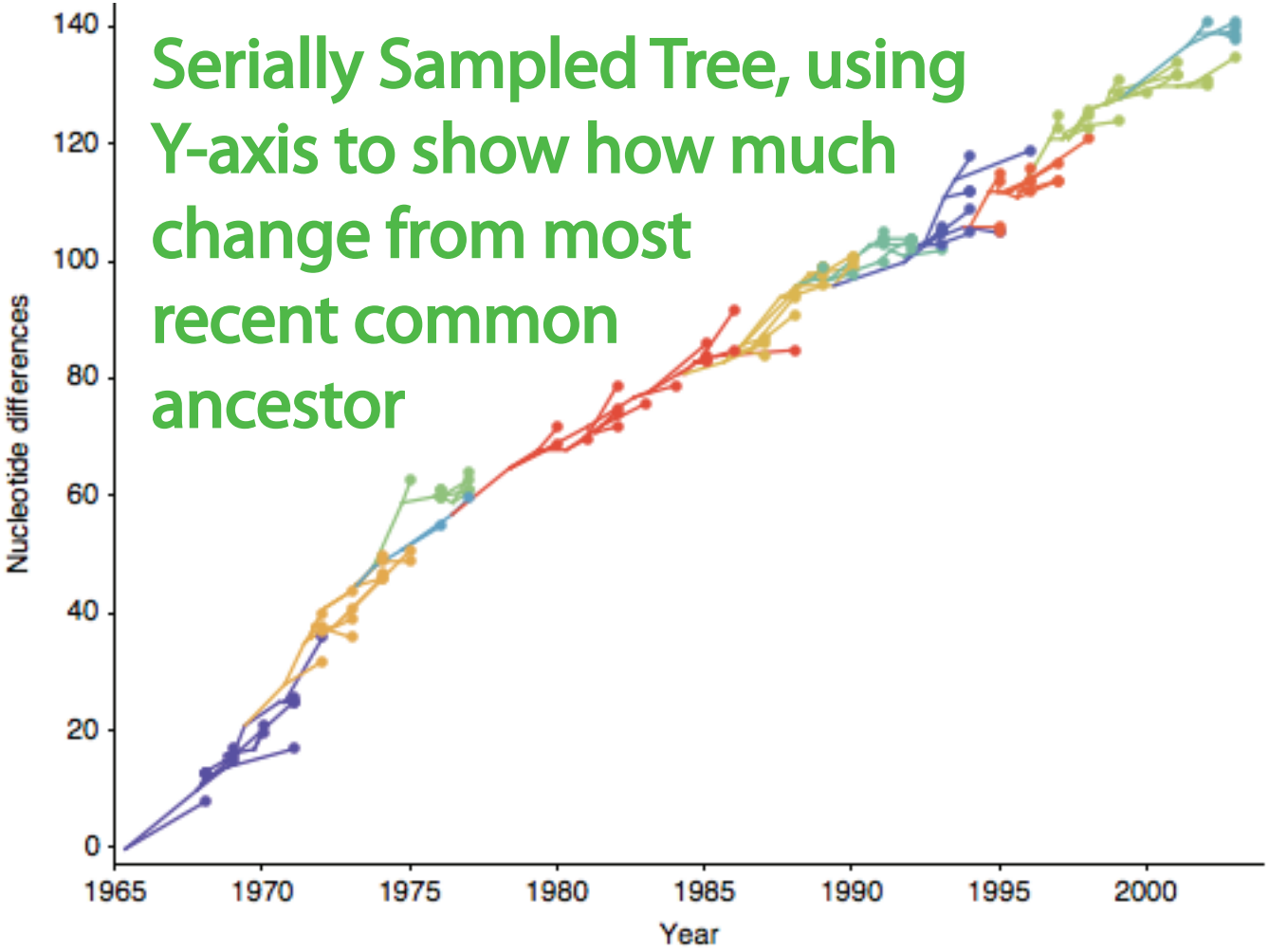


Figure 2 from
Smith et al. 2004.
Science 305:371-376

"... Comparison of antigenic and genetic evolution of influenza A virus.
(A) Phylogenetic tree of the HA1 nucleotide sequences, color-coded based on antigenic clusters of Fig. 1. ...
(B) Genetic map of the HA1 amino acid sequences, color-coded according to the antigenic clusters of Fig. 1. The vertical and horizontal axes represent genetic distance...
(C) The same antigenic map of influenza A virus strains as shown in Fig. 1, except ... Arrows indicate the two cluster transitions for which the amino acid substitution N145K is the only cluster-difference substitution (Table 1, fig. S1). ..."

Two ways of looking at HA1 evolution in H3N2

Serially Sampled Tree, using Y-axis to show how much change from most recent common ancestor



both figures taken from
http://www.trevorbedford.com/writeups/interpretable_phylogenies.html

Serially Sampled Tree

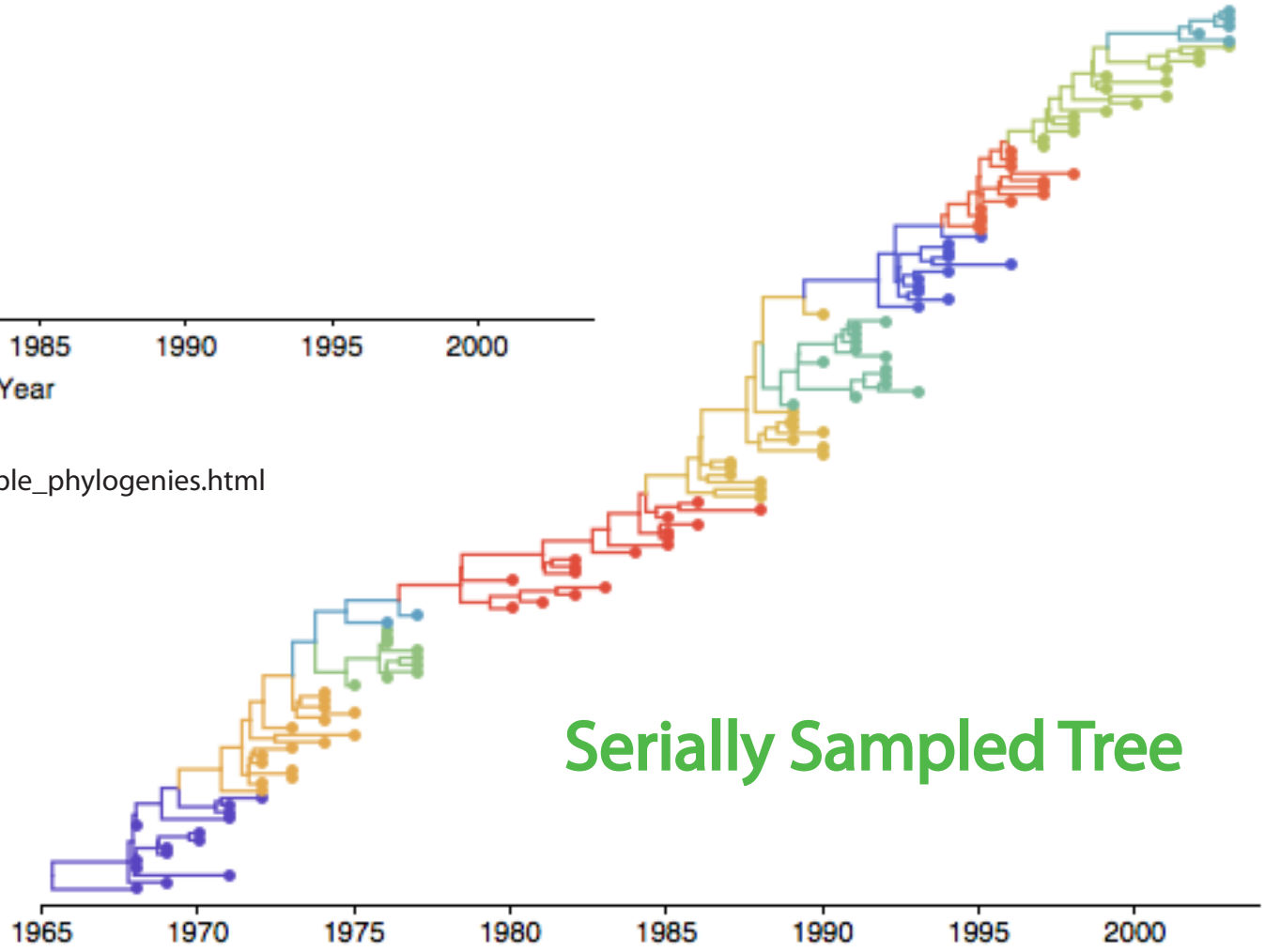


Figure 2A from Russell et al. 2008. 320:340-346
Shows estimated times when different antigenic types of Influenza A arise relative to the "middle" time among regions

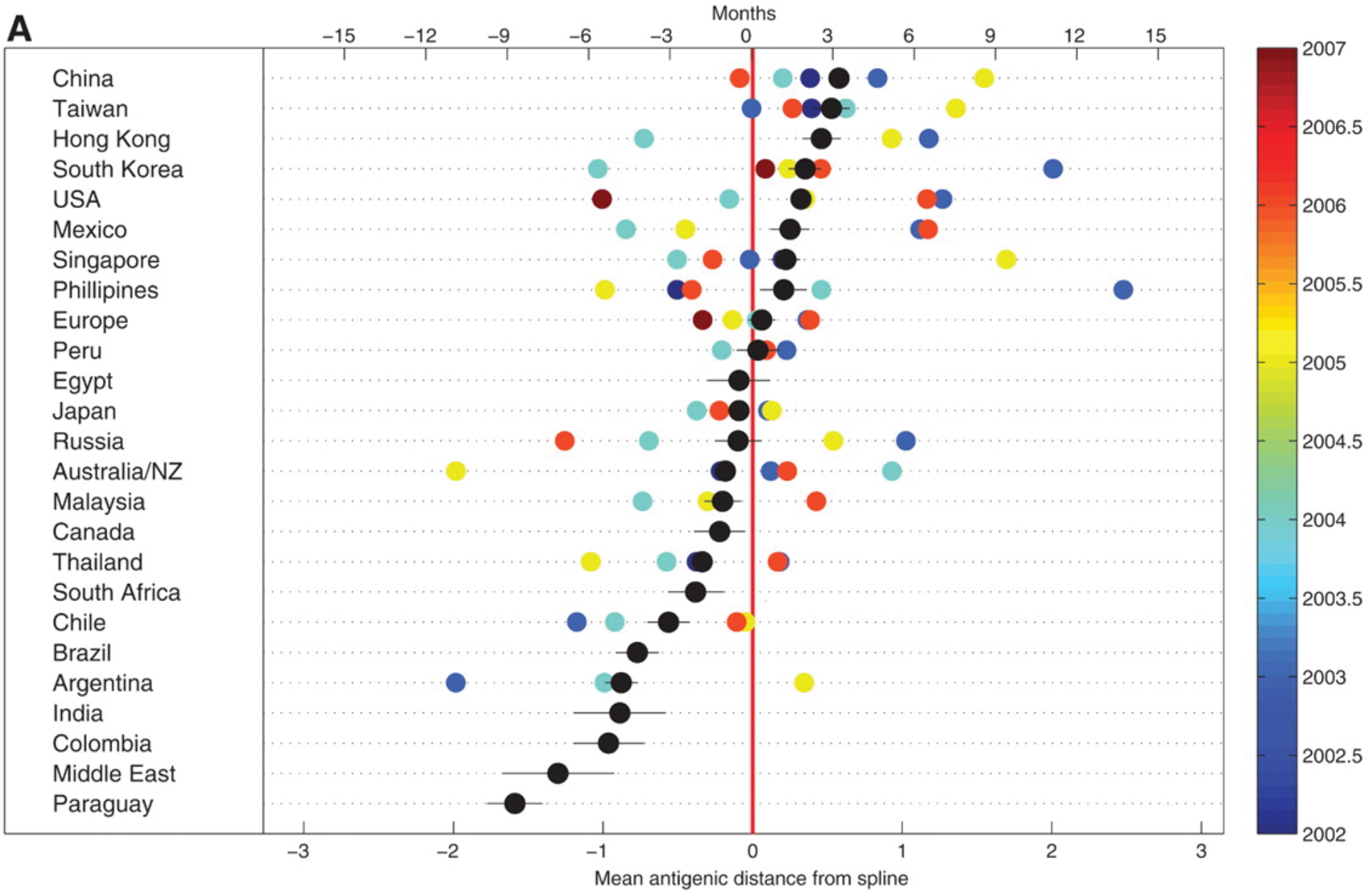
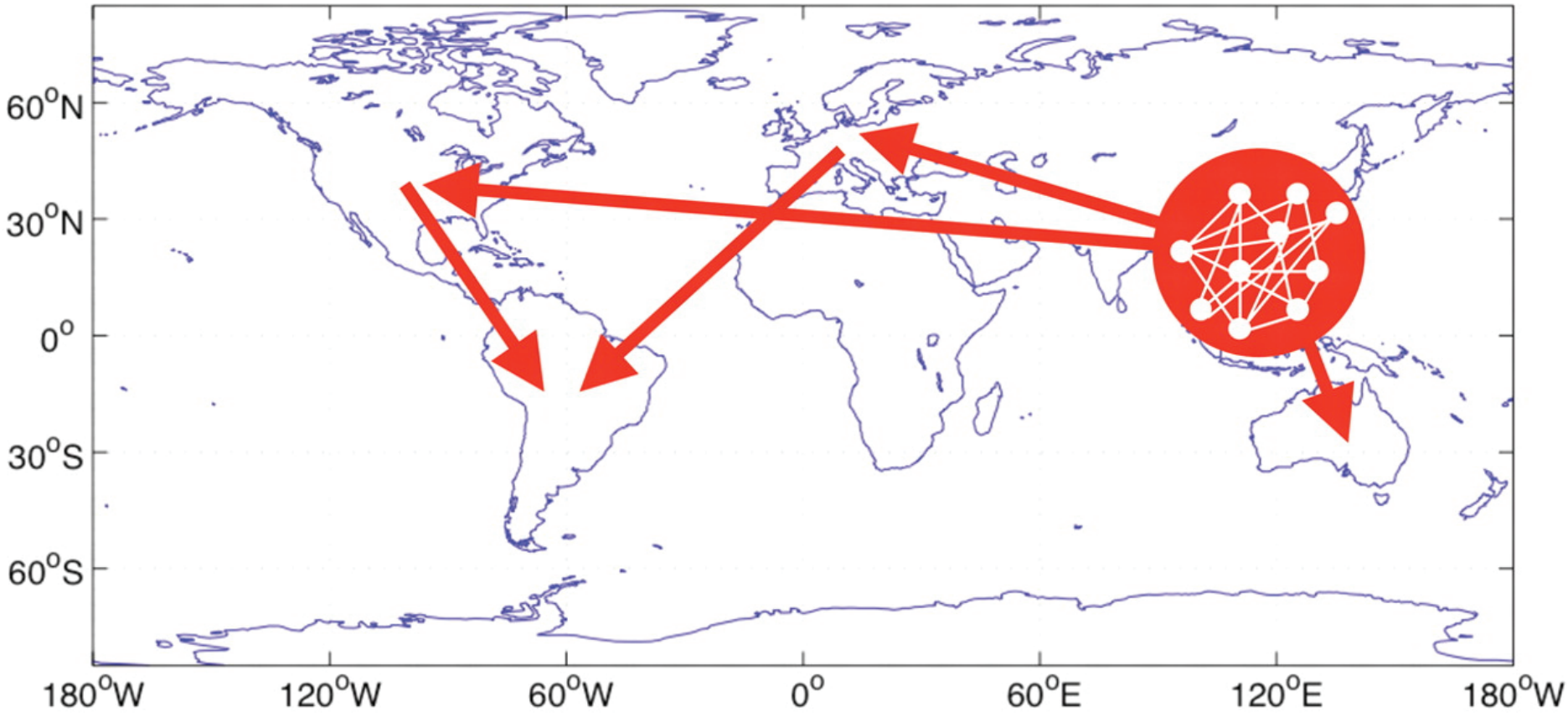
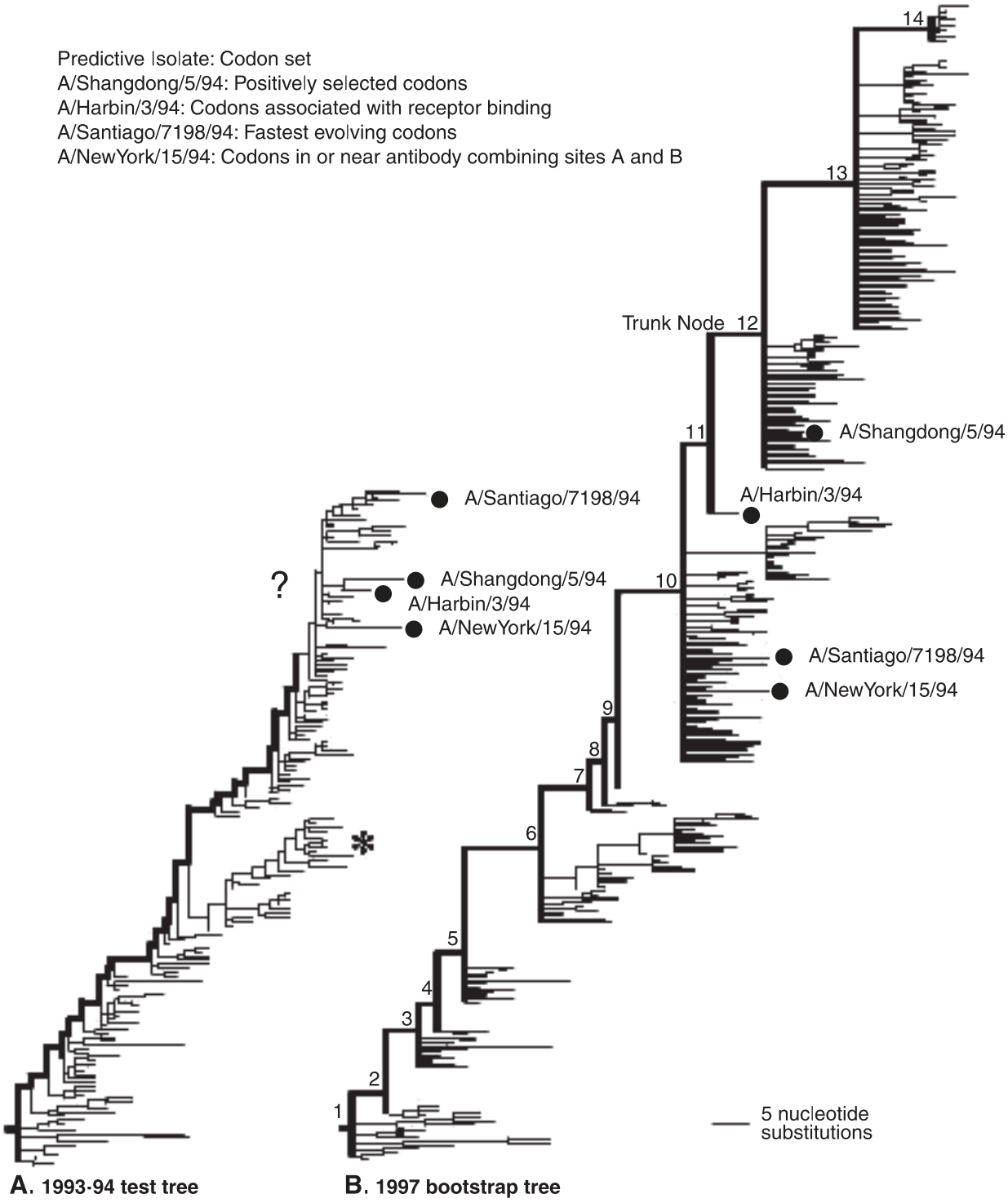


Figure 5 from Russell et al. 2008. 320:340-346

Shows inferred patterns of Influenza origination and circulation



Can evolution be used to inform vaccine design?



Bush et al. 1999.
Science 286:1921-1925

Benefits of Flu Vaccines

"[D]uring 2016-2017, flu vaccination prevented an estimated 85,000 flu-related hospitalizations. ...

A 2018 study showed that from 2012 to 2015, flu vaccination among adults reduced the risk of being admitted to an intensive care unit (ICU) with flu by 82 percent. ...

Flu vaccination has been shown in several studies to reduce severity of illness in people who get vaccinated but still get sick. ... A 2018 study showed that among adults hospitalized with flu, vaccinated patients were 59 percent less likely to be admitted to the ICU than those who had not been vaccinated. Among adults in the ICU with flu, vaccinated patients on average spent 4 fewer days in the hospital than those who were not vaccinated. ...

Getting vaccinated yourself may also protect people around you, including those who are more vulnerable to serious flu illness, like babies and young children, older people, and people with certain chronic health conditions."

from https://www.cdc.gov/flu/about/qa/vaccineeffect.htm

Flu vaccines typically constrained to protect against one B strain and two A strains. (Takes about 6 months to mass produce flu vaccine)

Sometimes, ineffective flu vaccine may be due to circulating strains differing from strain used for vaccine. This could be due to wrong predictions about which flu strain will predominate by time at which vaccine can be widely administered. Or, could be due to evolution in flu strain ("antigenic drift") following time at which vaccine designed.

In 2012-2013, low vaccine effectiveness for H3N2 flu type apparently due to way vaccine is produced in chicken eggs. Specifically, survival of virus in eggs may be favored by mutations that occur in the eggs.

Most vaccine in U.S. produced by growing virus in chicken eggs, then killing virus and purifying antigens (flu shots) or weakening virus (nasal spray).

Technologies to avoid the chicken egg step exist and others are being developed ...

There are three different influenza vaccine production technologies approved by the U.S. Food and Drug Administration (FDA): egg-based flu vaccine, cell-based flu vaccine, and recombinant flu vaccine.

...

Egg-Based Flu Vaccines: The most common way that flu vaccines are made is using an egg-based manufacturing process that has been used for more than 70 years. Egg-based vaccine manufacturing is used to make both inactivated (killed) vaccine (usually called the "flu shot") and live attenuated (weakened) vaccine (usually called the "nasal spray flu vaccine").

quoted from https://www.cdc.gov/flu/protect/vaccine/how-fluvaccine-made.htm

"Cell-based flu vaccine production does not require chicken eggs because the vaccine viruses used to make vaccine are grown in animal cells. Cell culture technology has the potential for a faster start-up of the flu vaccine manufacturing process. The process of creating cell-based flu vaccines involves several steps. First, the World Health Organization (WHO) recommends cell-grown CVVs for distribution to manufacturers. Next, manufacturers inoculate the CVVs into cultured mammalian cells (instead of into eggs) and allow them to replicate for a few days. Then, the virus-containing fluid is collected from the cells and the virus antigen is purified. The manufacturing process continues with purification and testing. "

CVV = Candidate Vaccine Virus

quoted from https://www.cdc.gov/flu/protect/vaccine/how-fluvaccine-made.htm

Recombinant Flu Vaccines [do] not require an egg-grown vaccine virus and [do] not use chicken eggs at all in the production process. Instead, manufacturers isolate a certain gene (the hemagglutinin or "HA" gene) from a naturally occurring ("wild type") recommended vaccine virus. This HA gene is then combined with portions of another virus that grows well in insect cells. ... The flu HA protein is then harvested from these cells and purified. The purified protein is packaged while waiting for FDA testing and approval to release lots. ...

This process can produce vaccine in the shortest amount of time because it is not limited by the selection of vaccine viruses that are adapted for growth in eggs or the development of cell-based vaccine viruses. "

[other recombinant production techniques are also being developed]

quoted from https://www.cdc.gov/flu/protect/vaccine/how-fluvaccine-made.htm

"Why is flu vaccine typically less effective against influenza A(H3N2) viruses?

...

[T]he changes that have occurred in influenza A(H3N2) viruses have more frequently resulted in differences between the virus components of the flu vaccine and circulating influenza viruses (i.e., antigenic change) compared with influenza A(H1N1) and influenza B viruses. That means that between the time when the composition of the flu vaccine is recommended and the flu vaccine is delivered, H3N2 viruses are more likely than H1N1 or influenza B viruses to have changed in ways that could impact how well the flu vaccine works.

...

Growth in eggs is part of the production process for most seasonal flu vaccines. While all influenza viruses undergo changes when they are grown in eggs, changes in influenza A(H3N2) viruses tend to be more likely to result in antigenic changes compared with changes in other influenza viruses. These so-called "egg-adapted changes" are present in vaccine viruses recommended for use in vaccine production and may reduce their potential effectiveness against circulating influenza viruses. "

from https://www.cdc.gov/flu/about/qa/vaccineeffect.htm

Next 3 pages taken from ...

http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM497026.pdf

(Unfortunately, this page seems to have disappeared from internet!)

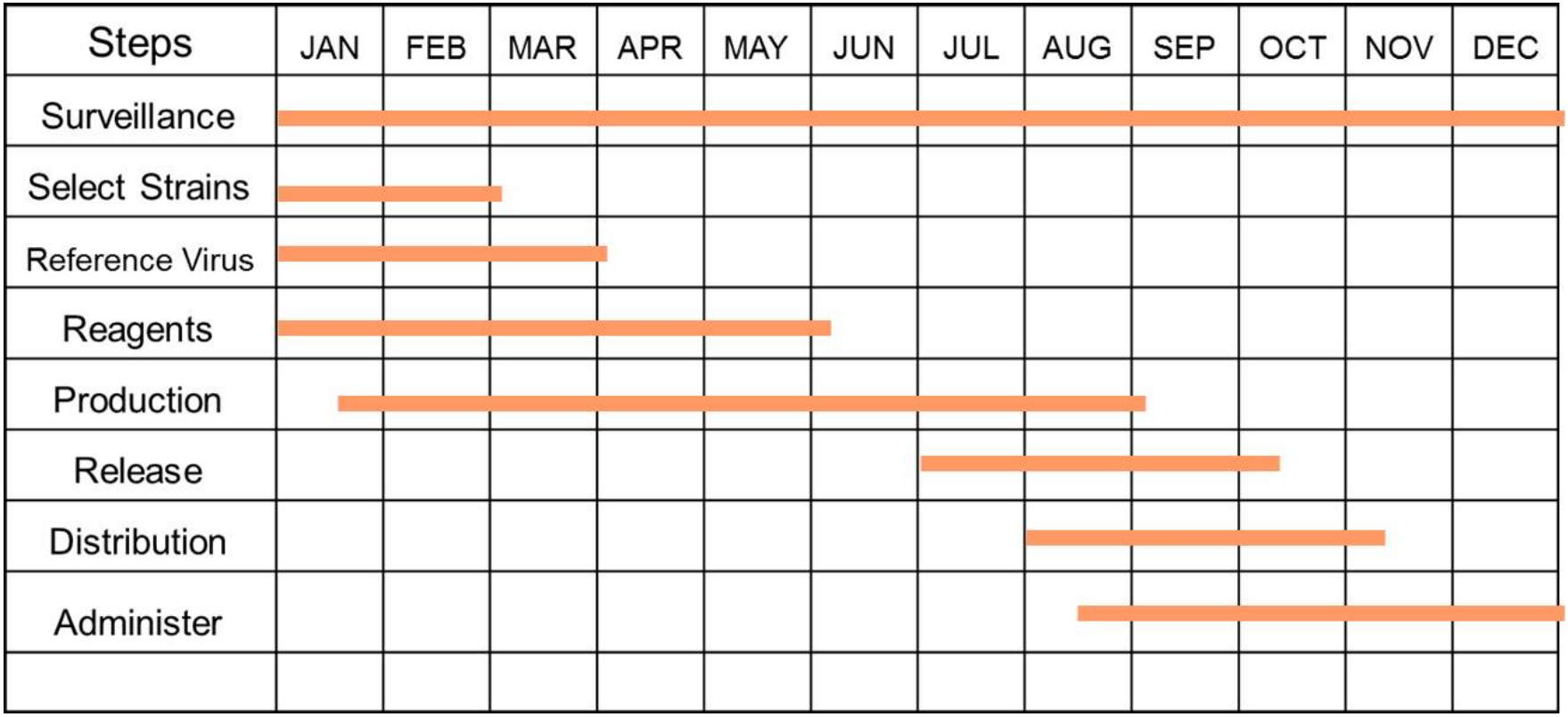
Types of Analyses Used for Vaccine Strain Selection

- Epidemiology of circulating strains *(CDC)*
  - Surveillance data from U.S. and around the world
- Antigenic relationships among contemporary viruses and candidate vaccine strains *(CDC/DOD/CBER)*
  - Hemagglutination inhibition (HI) tests using post-infection ferret sera
  - HI tests using panels of sera from humans receiving recent inactivated influenza vaccines
  - Virus neutralization tests
  - Antigenic cartography
  - Phylogenetic analyses of HA and NA genes
  - Vaccine effectiveness

Key Challenges for Vaccine Strain Selection

- Vaccine effectiveness depends on match between the hemagglutinin (HA) of the vaccine and the HA of circulating strains of virus
  - Antigenic drift of HA continuous for influenza A and B
  - Antibody to HA correlated with vaccine efficacy
- Timelines for influenza vaccine production are relatively fixed
  - Strain selection in February/March necessary for availability of vaccine for subsequent northern hemisphere winter (influenza season)
  - Manufacturers typically begin production of monovalent of one strain before strain selection recommendations are made (at risk)
- Availability of reference strains (candidate vaccine viruses) suitable for vaccine manufacture
  - Vaccine production depends on growth properties of strains used for manufacture
  - Strain-specific reagents needed for potency determination (inactivated and recombinant protein vaccines)

Seasonal Influenza Vaccine Production Timetable



Estimated Vaccine Effectiveness for recent flu seasons:

```
2004-05: 10% (-36 to 40%)
2005-06: 21% (-52 to 59%)
2006-07: 52% ( 22 to 70%)
2007-08: 37% ( 22 to 49%)
2008-09: 41% ( 30 to 50%)
2009-10: 56% ( 23 to 75%)
2010-11: 60% ( 53 to 66%)
2011-12: 47% ( 36 to 56%)
2012-13: 49% ( 43 to 55%)
2013-14: 52% ( 44 to 59%)
2014-15: 19% ( 10 to 27%)
2015-16: 48% ( 41 to 55%)
2016-17: 40% ( 32 to 46%)
2017-18: 38% ( 31 to 43%)
2018-19: 29% ( 21 to 35%)
2019-20: 39% ( 32 to 44%)
2020-21: Not enough data to estimate!
2021-22: 36% ( 21 to 48%)
```

from https://www.cdc.gov/flu/vaccines-work/past-seasons-estimates.html
(and from now unavailable similar cdc pages in previous years)

Vaccine Effectiveness is % Flu Cases fewer in vaccinated population relative to unvaccinated population.

"... FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) met in Silver Spring, Maryland, on March 5, 2021, to select the influenza viruses for the composition of the influenza vaccine for the 2021-2022 U.S. influenza season. During this meeting, the advisory committee reviewed and evaluated the surveillance data related to epidemiology and antigenic characteristics of recent influenza isolates, serological responses to 2020-2021 vaccines, and the availability of candidate strains and reagents.

Influenza virus strains were selected based on the influenza vaccine production method; egg-based and cell- or recombinant based.

The committee recommended that the quadrivalent formulation of egg-based influenza vaccines for the U.S. 2021-2022 influenza season contain the following:

an A/Victoria/2570/2019 (H1N1) pdm09-like virus;
an A/Cambodia/e0826360/2020 (H3N2)-like virus;
a B/Washington/02/2019- like virus (B/Victoria lineage);
a B/Phuket/3073/2013-like virus (B/Yamagata lineage). ..."
[Note: the 4th/final component has not changed for at least several years]

from https://www.fda.gov/vaccines-blood-biologics/lot-release/influenza-vaccine-2021-2022-season

Voting Questions for the Committee

1) For the influenza A (H1N1) component of the 2022-2023 influenza virus vaccines in the U.S., does the committee recommend:

- an A/Victoria/2570/2019 (H1N1)pdm09-like virus (Egg-based Vaccines)
- an A/Wisconsin/588/2019 (H1N1)pdm09-like virus (Cell- or Recombinant-based Vaccines)

2) For the influenza A (H3N2) component of the 2022-2023 influenza virus vaccine in the U.S., does the committee recommend:

- an A/Darwin/9/2021 (H3N2)-like virus (Egg-based Vaccines)
- an A/Darwin/6/2021 (H3N2)-like virus (Cell- or Recombinant-based Vaccines)

3) For the influenza B component of the 2022-2023 trivalent and quadrivalent influenza virus vaccines in the U.S., does the committee recommend inclusion of a B/Austria/1359417/2021-like virus (B/Victoria lineage)

4) For quadrivalent 2022-2023 influenza vaccines in the U.S., does the committee recommend inclusion of a B/Phuket/3073/2013-like virus (B/Yamagata lineage) as the 2nd influenza B strain in the vaccine

Some Viral Epidemic Web Pages to see:

fluview:

https://www.cdc.gov/flu/weekly/fluviewinteractive.htm

NextStrain: https://nextstrain.org

Virological.org: http://virological.org