



DEPARTMENT OF THE AIR FORCE
59TH MEDICAL WING (AETC)
JOINT BASE SAN ANTONIO - LACKLAND TEXAS

8 APR 2016

MEMORANDUM FOR SGVU
ATTN: THOMAS GIBBONS

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled **The Influenza Virus and the 2009 H1N1 Outbreak** presented at **2016 Texas Association for Clinical Laboratory Science Conference, San Antonio, TX 6-8 April 2016** with MDWI 41-108, and has been assigned local file #**16146**.
2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.
3. Please know that if you are a Graduate Health Sciences Education student and your department has told you they cannot fund your publication, the 59th Clinical Research Division may pay for your basic journal publishing charges (to include costs for tables and black and white photos). We cannot pay for reprints. If you are 59 MDW staff member, we can forward your request for funds to the designated wing POC.
4. Congratulations, and thank you for your efforts and time. Your contributions are vital to the medical mission. We look forward to assisting you in your future publication/presentation efforts.

A handwritten signature in black ink, appearing to read "Linda Steel-Goodwin", is positioned above the typed name.

LINDA STEEL-GOODWIN, Col, USAF, BSC
Director, Clinical Investigations & Research Support

PROCESSING OF PROFESSIONAL MEDICAL RESEARCH PUBLICATIONS/PRESENTATIONS

TO: Clinical Research Division/SGVU (59 MDW/SGVU)	FROM: Author's Name, Rank, Grade, Office Symbol Thomas Gibbons, GS-14, 59 MDW/SGVUL	PROTOCOL NUMBER: N/A
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PROTOCOL TITLE - [NOTE: For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.]
 Educational Presentation

1. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED

The Influenza Virus and the 2009 H1N1 Outbreak

2. FUNDING RECEIVED FOR THIS STUDY? YES NO **FUNDING SOURCE:**

3. IS THIS MATERIAL CLASSIFIED? YES NO

4. IS THIS MATERIAL SUBJECT TO ANY LEGAL RESTRICTIONS FOR PUBLICATION OR PRESENTATION THROUGH A COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA), MATERIAL TRANSFER AGREEMENT (MTA), INTELLECTUAL PROPERTY RIGHTS AGREEMENT ETC.? YES NO
 NOTE: If the answer is "YES" then attach a copy to the Agreement to the Publications/Presentations Request Form.

5. MATERIAL IS FOR (Check appropriate box or boxes for approval with this request.) (ATTACH COPY OF MATERIAL TO BE PUBLISHED/PRESENTED) DOMESTIC RELEASE FOREIGN RELEASE

<input type="checkbox"/>	PUBLICATION/JOURNAL (List intended publication/journal)
<input type="checkbox"/>	PUBLICATION ABSTRACT (List intended journal.)
<input type="checkbox"/>	POSTER (To be demonstrated at meeting/Name of Meeting, City, State, and Date of Meeting)
<input checked="" type="checkbox"/>	PLATFORM PRESENTATION (At civilian institutions/Name of Meeting, State, Date of Meeting) 2016 Texas Association For Clinical Laboratory Science Conference, San Antonio Texas, April 6-8 2016.
<input type="checkbox"/>	OTHER (Describe: Name of Meeting, City, State, and Date of Meeting)

6. WHAT IS THE EXPECTED DATE YOUR PRESENTATION/PUBLICATION WILL BE SUBMITTED TO THE DEFENSE TECHNICAL INFORMATION CENTER (DTIC)?

POINT OF CONTACT

7. WHO IS THE PRIMARY 59 MDW POINT OF CONTACT? (Last, First, MI.) (Include email) Gibbons, Thomas, F. thomas.gibbons.4@us.af.mil	DUTY PHONE/PAGER No. 210-292-7363
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AUTHORSHIP AND CO-AUTHOR(S) (List in the order they will appear in the manuscript)

LAST NAME, FIRST NAME AND MI.	GRADE/RANK	SQUADRON/GROUP/OFFICE SYM	INSTITUTION (if not 59 MDW)
a. Primary/corresponding author Gibbons, Thomas F.	GS-14	59 MDW/SGVUL	
b.			
c.			
d.			
e.			
f.			
g.			

I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401_IP AND 59 MDWI 41-108. I HAVE READ THE FINAL VERSION OF THE ATTACHED MATERIAL AND CERTIFY THAT IT IS AN ACCURATE MANUSCRIPT FOR PUBLICATION AND/OR PRESENTATION.

AUTHOR'S PRINTED NAME/RANK/GRADE Thomas F. Gibbons GS-14	AUTHOR'S SIGNATURE GIBBONS.THOMAS.F.1128005281 <small>Digitally signed by GIBBONS.THOMAS.F. 1128005281 DN: cn=US, o=U.S. Government, ou=DoD, ou=AF, ou=59AF, c=GIBBONS.THOMAS.F. 1128005281 Date: 2016.03.28 18:24:00 -0500</small>	DATE Mar 28, 2016
APPROVING AUTHORITY'S PRINTED NAME, RANK, TITLE Paul T. Barnicott GS-15	APPROVING AUTHORITY'S SIGNATURE BARNICOTT.PAUL.T.1102318273 <small>Digitally signed by BARNICOTT.PAUL.T. 1102318273 DN: cn=US, o=U.S. Government, ou=DoD, ou=AF, ou=59AF, c=BARNICOTT.PAUL.T. 1102318273 Date: 2016.03.28 18:24:00 -0500</small>	DATE Mar 29, 2016

PROCESSING OF PROFESSIONAL MEDICAL RESEARCH PUBLICATIONS/PRESENTATIONS

1st INDORSEMENT (SGVU Use Only)

TO: Clinical Research Division (59 MDW/SGVU) (Contact 292-7141 for email instructions)	1. DATE RECEIVED	2. ASSIGNED PROCESSING REQUEST FILE NUMBER
	3/29/2016	16146

3. DATE REVIEWED 30 Mar 2016	4. DATE FORWARDED TO PA
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5. AUTHOR CONTACTED FOR RECOMMENDED OR NECESSARY CHANGES
 NO YES If yes give date: N/A

6. COMMENTS
 APPROVED DISAPPROVED

The presentation is approved. This will require a JA Ethics review.

PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER Rocky Calcote, PhD, Clinical Research Administrator	DATE	SIGNATURE OF REVIEWER CALCOTE.ROCKY.D.1178245844 <small>Digitally signed by CALCOTE.ROCKY.D.1178245844 DN: c=US, ou=U.S. Government, ou=DoD, ou=PKI, ou=USAF, cn=CALCOTE.ROCKY.D.1178245844 Date: 2016.03.30 07:41:33 -0500</small>
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2nd INDORSEMENT (PA Use Only)

TO: 59 MDW OFFICE OF PUBLIC AFFAIRS (PA)	1. DATE RECEIVED	2. DATE FORWARDED TO 59 MDW/SGVU
	Apr 6, 2016	Apr 7, 2016

6. COMMENTS APPROVED (In compliance with security and policy review directives.) DISAPPROVED

PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER Christopher Carwile, TSgt/E-6, NCOIC, PA	DATE Apr 7, 2016	SIGNATURE OF REVIEWER CARWILE.CHRISTOPHER.S TEWART.1280477229 <small>Digitally signed by CARWILE.CHRISTOPHER.STEWART.1280477229 DN: c=US, ou=U.S. Government, ou=DoD, ou=PKI, ou=USAF, cn=CARWILE.CHRISTOPHER.STEWART.1280477229 Date: 2016.04.07 12:53:50 -0500</small>
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3rd INDORSEMENT (SGVU Use Only)

TO: 59 MDW/SGVU	1. DATE RECEIVED
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2. SENIOR AUTHOR NOTIFIED BY PHONE OF APPROVAL OR DISAPPROVAL: YES NO Could not be reached Left message

3. DATE WRITTEN NOTICE OF APPROVAL AND CLEARANCE MAILED TO AUTHOR: _____

4. COMMENTS APPROVED DISAPPROVED

PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	SIGNATURE OF REVIEWER	DATE
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DEPARTMENT OF THE AIR FORCE
AIR EDUCATION AND TRAINING COMMAND

1 April 2016

MEMORANDUM FOR 59 MDW/SGVU

FROM: 502 ISG/JAC

SUBJECT: Ethics Review for Presentation Approval Request

1. The poster presentation titled "The Influenza Virus and the 2009 H1N1 Outbreak" has been submitted for legal review. There are no apparent conflicts of interests and the poster presentation includes the required disclaimer. This poster presentation may be submitted for exhibition at conference.
2. **FACTS:** The poster presentation titled "The Influenza Virus and the 2009 H1N1 Outbreak" was submitted by Dr. Thomas Gibbons of 59 MDW/SGUVL. Dr. Gibbons plans to present this poster presentation at the 2016 Texas Association for Clinic Laboratory Science Conference in San Antonio, Texas from 6 – 8 April 2016.
3. **LAWS AND REGULATIONS:** DoD 5500.07-R, Joint Ethics Regulation (JER), section 3-305 lays out rules governing "Teaching, Speaking and Writing." If the abstract will include the presenter's rank or title and "deal in significant part with any ongoing or announced policy, program or operation" of the Air Force, the presenter is required to include a disclaimer that states the "views presented are those of the speaker or author and do not necessarily represent the views of DoD or its Components."
4. **ANALYSIS:** While this poster presentation does not deal "in significant part with any ongoing or announced policy, program or operation" of the Air Force, the presenter's affiliation and/or rank will nevertheless be included in the poster presentation. Accordingly, the disclaimer is required here and Dr. Gibbon has properly included it. A Public Affairs review will be needed if it has not already been obtained. There are no apparent conflicts of interest that would prohibit presentation of this information at this professional meeting.
5. **CONCLUSION:** The poster presentation presented for review included the disclaimer required by the JER. There do not appear to be any conflicts of interest. If you have any questions, please call the undersigned at 671-3362.


MARK E. COON, Major, USAF
Acting Chief, Civil Law

The Influenza Virus and the 2009 H1N1 Outbreak

Thomas. F. Gibbons, Ph.D.
Chief, Laboratory Services Branch
Clinical Research Division
Wilford Hall Ambulatory Surgical Center

The views expressed are those of the presenter and do not reflect the official policy of the Department of Defense or its Components

Overview

- “The Story”
- Key aspects of the influenza virus
- Laboratory techniques utilized to discover the 2009 H1N1 influenza virus
- Laboratory techniques instrumental to influenza surveillance/vaccine contents

Video depicting USAF “Epi lab’s” discovery of H1N1

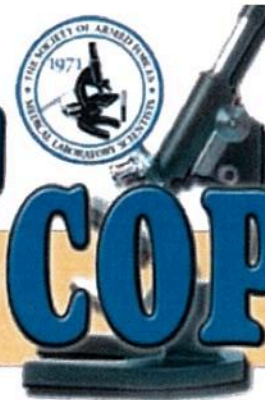
April 1 2009 Video team comes to our lab to film a documentary on our role with respect to influenza surveillance

<http://www.ndep.us/Tracking-the-Flu>

April 16 2009 “Found a weird flu virus today”

SOCIETY SCOPE

PO BOX 2549 • FAIRFAX, VIRGINIA 22031-0549
Fall 2009 • Volume 12 Number 3



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USAFSAM Influenza Surveillance during the off season: April 2009 – October 2009

By: Thomas F. Gibbons, Maj, USAF,
BSC, Ph.D, MT (ASCP)
Chief, Molecular Diagnostics, USAFSAM

Influenza virus is a segmented negative stranded RNA virus composed of 8 gene segments belonging to the Orthomyxoviridae family. Three types of influenza have been detected, A, B and C, two of which (A and B) are a

http://www.safmls.org/society_scope.html

Volume 12, Issue 3

Key aspects of the influenza virus

Types of influenza

A,B, and C (Epidemics = A &B)
Pandemics = A only

1918 = H1N1
50,000,000 deaths

1957 = H2N2
1,000,000 deaths

Shift and Drift

1968 = H3N2
500,000 deaths

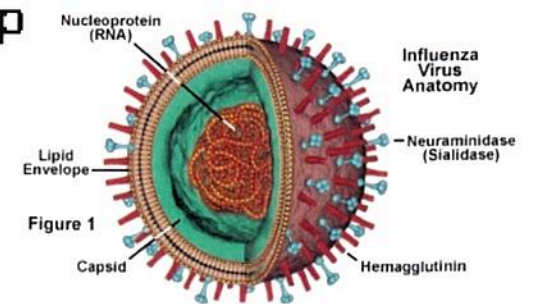
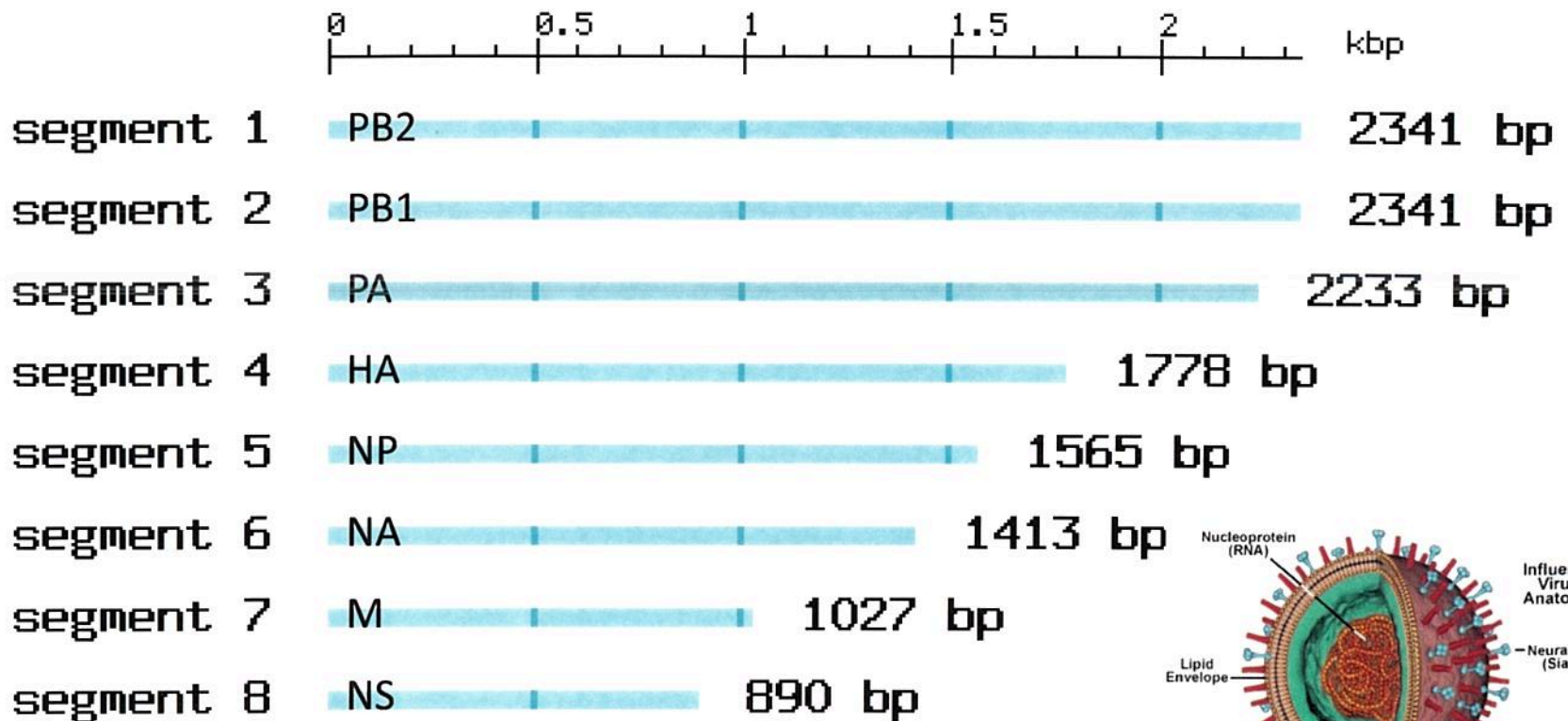
2009 H1N1

Annual flu
250,000 deaths

H5N1 annual
25 - 75

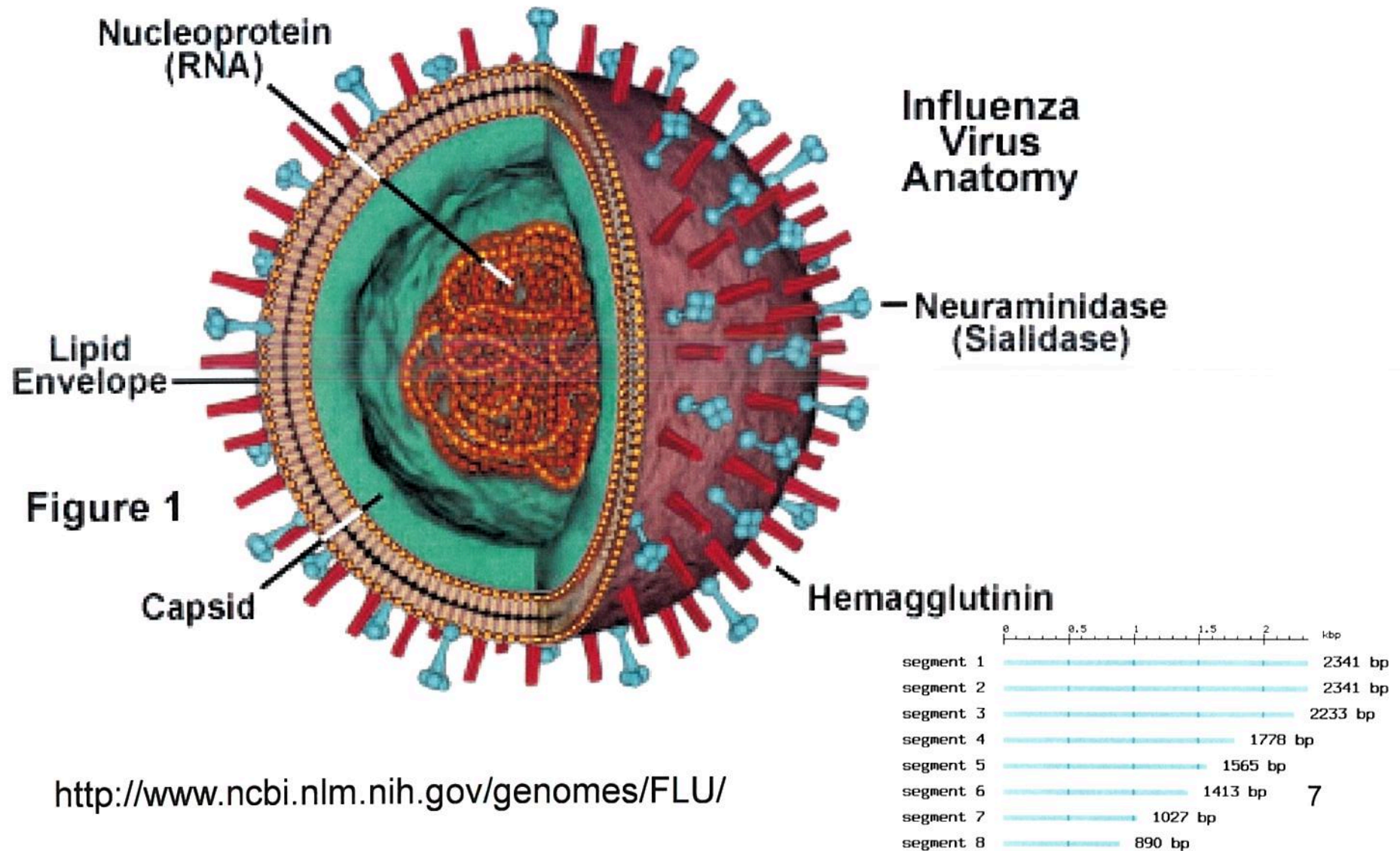
Key aspects of the influenza virus

Influenza Genome



Key aspects of the influenza virus

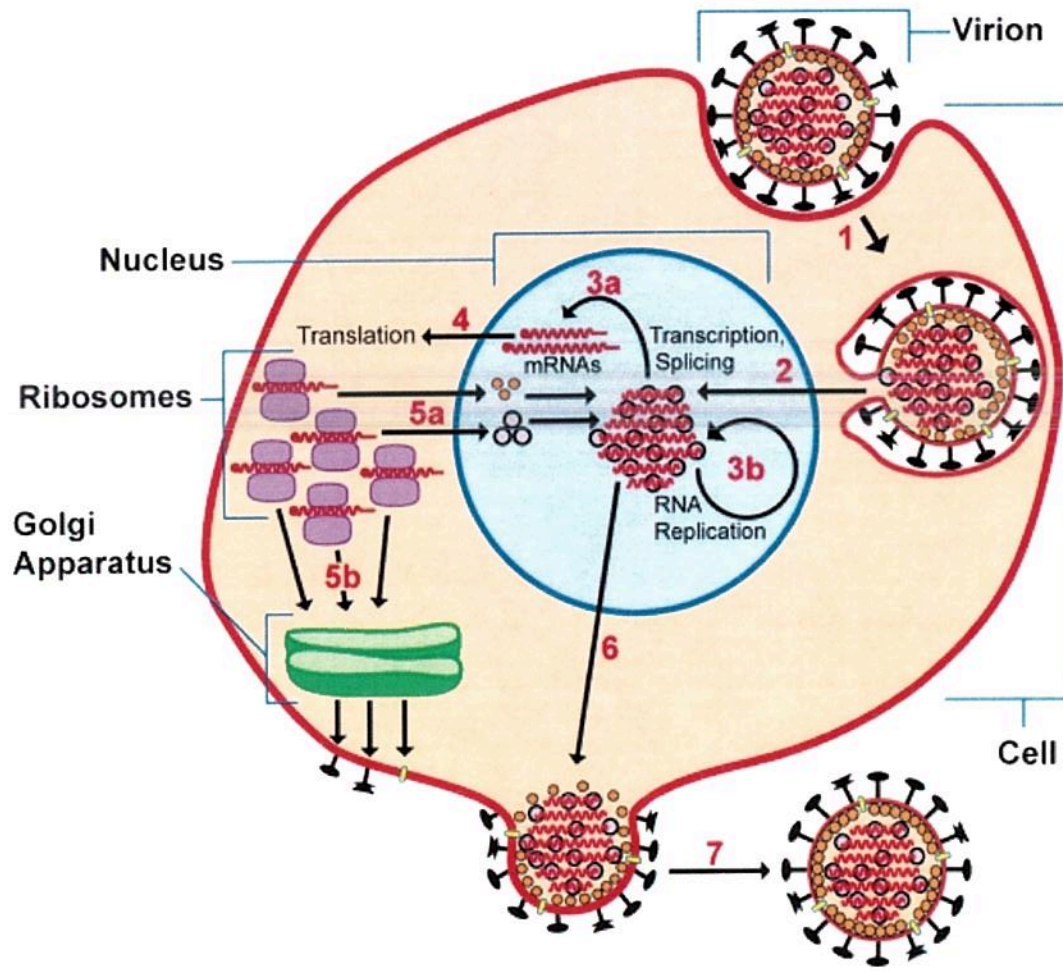
Influenza



<http://www.ncbi.nlm.nih.gov/genomes/FLU/>

Key aspects of the influenza virus

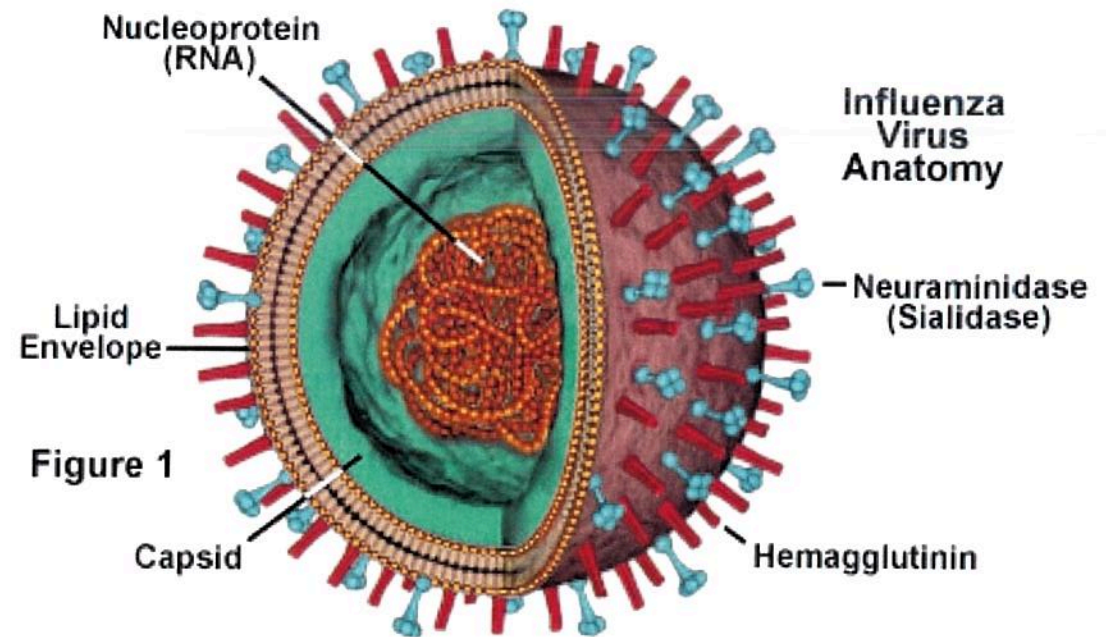
Influenza Life Cycle



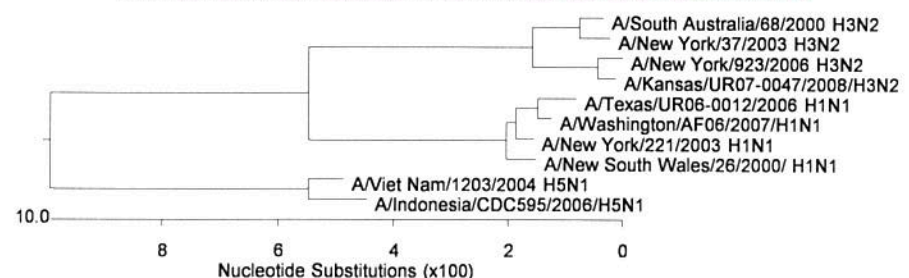
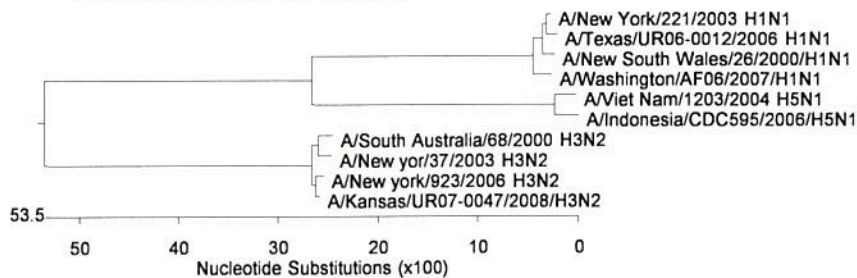
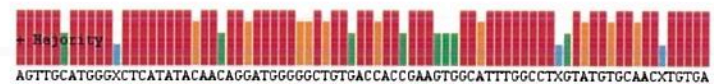
- 1: Binding and entry
- 2: Release of vRNPs
- 3: RNA replication
- 4: RNA translation
- 5: Protein transport
- 6: Viral transport
- 7: Viral budding and release

Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

- Lateral Flow assay
- Culture
- PCR
- Sequencing



Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus



Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

```
GACCAATCXTGTCACCTCTGACTAAGGGXATTTTAGGATTTGTGTTACGCTCACCGTGCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTXAATGG
60      170      180      190      200      210      220      230      240      250      260      270
GACCAATCCTTGTACCTCTGACTAAGGGAATTTTAGGATTTGTGTTACGCTCACCGTGCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTAAAATGG
GACCAATCCTTGTACCTCTGACTAAGGGGATTTTAGGATTTGTGTTACGCTCACCGTGCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTTAATGG
```

Matrix Gene

Hemagglutinin Gene

```
GXGCTATAAACAXCAGXCTXCCXTTTCAGAAAXTACAXCCXTCACAATXGGAXAXTGTCCAAAXTATGTXAXXAGXXCAAAATTXAGXXTGGYXACAGGAXTXAGGAAXATCCCXTC
920      930      940      950      960      970      980      990      1000      1010      1020      1030
GTGCTATAAACACCAGCCTCCCATTTCAGAAATATACATCCGATCACAATTGGAAAATGTCCAAAATATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATATCCCGTC
GAGCTATAAACAGCAGTCTTCCTTCCAGAACGTACACCCAGTCACAATAGGAGAGTGTCCAAAGTATGTCCAGGAGTGCAAAATTAAGGATGGTTACAGGACTAAGGAACATCCCATC
```



Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

A/California/04/2009 (H1N1)

PB2: Swine 97.6%, Duck 97.2%, Human 96.8%

PB1: Swine 97.0%, Turkey 96.6%

PA: Swine 96.0%, Human 95.8%

HA: Swine 96.4%, Turkey 96.2%

NP: Swine 97.8%

NA: Swine 93.6%

MA: Swine 98.2%

NS: Swine 95.6%

What is the swine flu like?

**blast analysis results for a recent
“Swine Flu”**

Blast results say “Swine”

but it is infecting humans

Blast comparison of first 500 nucleotides for each segment

Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

A/Washington/AF06/2007 (H1N1)

PB2: Human 99.8%

PB1: Human 100%

PA: Human 99.6%

HA: Human 99.6%

NP: Human 99.6%

NA: Human 99.8%

MA: Human 100%

NS: Human 100%

What is the human H1N1 flu like?

blast analysis results for one of the recent "Human Flu"

Blast comparison of first 500 nucleotides for each segment

Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

Gene by gene comparison of “Swine” H1N1 with “Human” H1N1

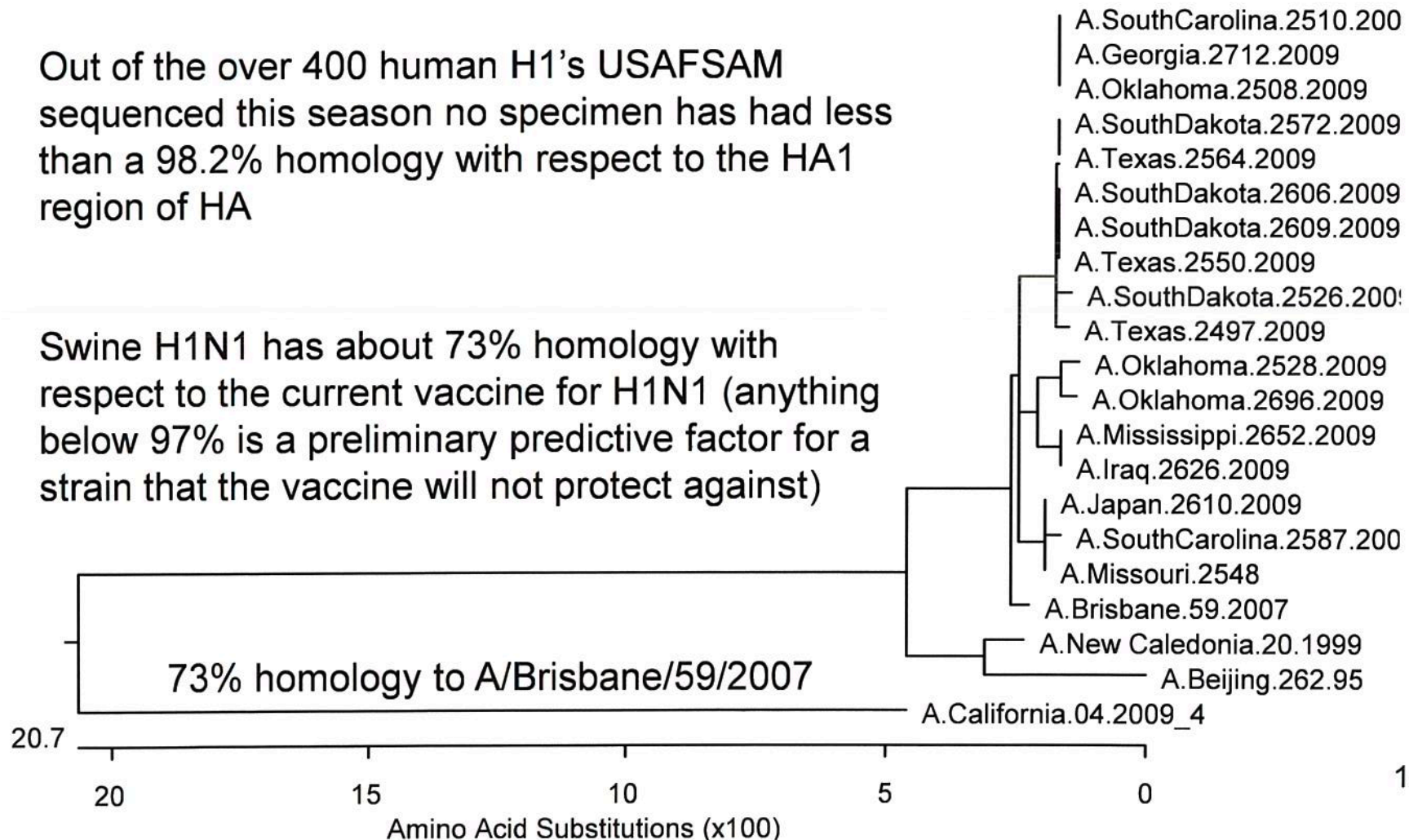
A/California/04/2009	% Homology	A/Washington/AF06/2007
PB2	82.7%	PB2
PB1	80.4%	PB1
PA	82.4%	PA
HA	75.9%	HA
NP	84.2%	NP
NA	78.0%	NA
MP	87.4%	MP
NS	81.7%	NS

Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

Phylogenetic Tree

Out of the over 400 human H1's USAFSAM sequenced this season no specimen has had less than a 98.2% homology with respect to the HA1 region of HA

Swine H1N1 has about 73% homology with respect to the current vaccine for H1N1 (anything below 97% is a preliminary predictive factor for a strain that the vaccine will not protect against)



Key laboratory techniques instrumental to influenza surveillance/vaccine contents

- Contents of the annual Flu Vaccine

- It is recommended that trivalent vaccines for use in the 2016-2017 northern hemisphere influenza season contain:

- **A/California/7/2009 (H1N1)pdm09-like virus;**

- **A/Hong Kong/4801/2014 (H3N2)-like virus;**

- **B/Brisbane/60/2008-like virus.**

- It is recommended that quadrivalent vaccines containing two influenza B viruses contain the above three viruses and a B/Phuket/3073/2013-like virus

Key laboratory techniques instrumental to influenza surveillance/vaccine contents

Host of origin/geographic location of first isolation/strain number/year of isolation

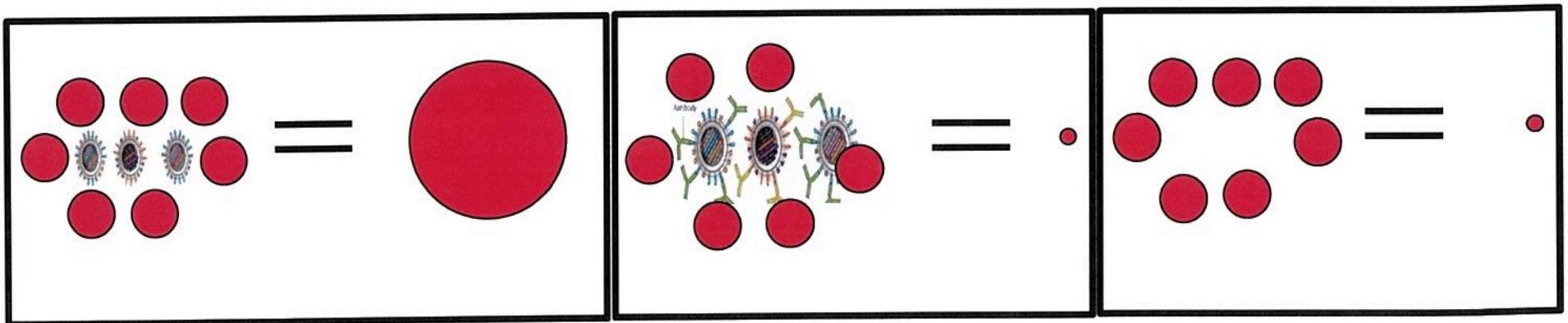
A/New York/11/2003 (H3N2)

A/chicken/Indonesia/R132-134/03

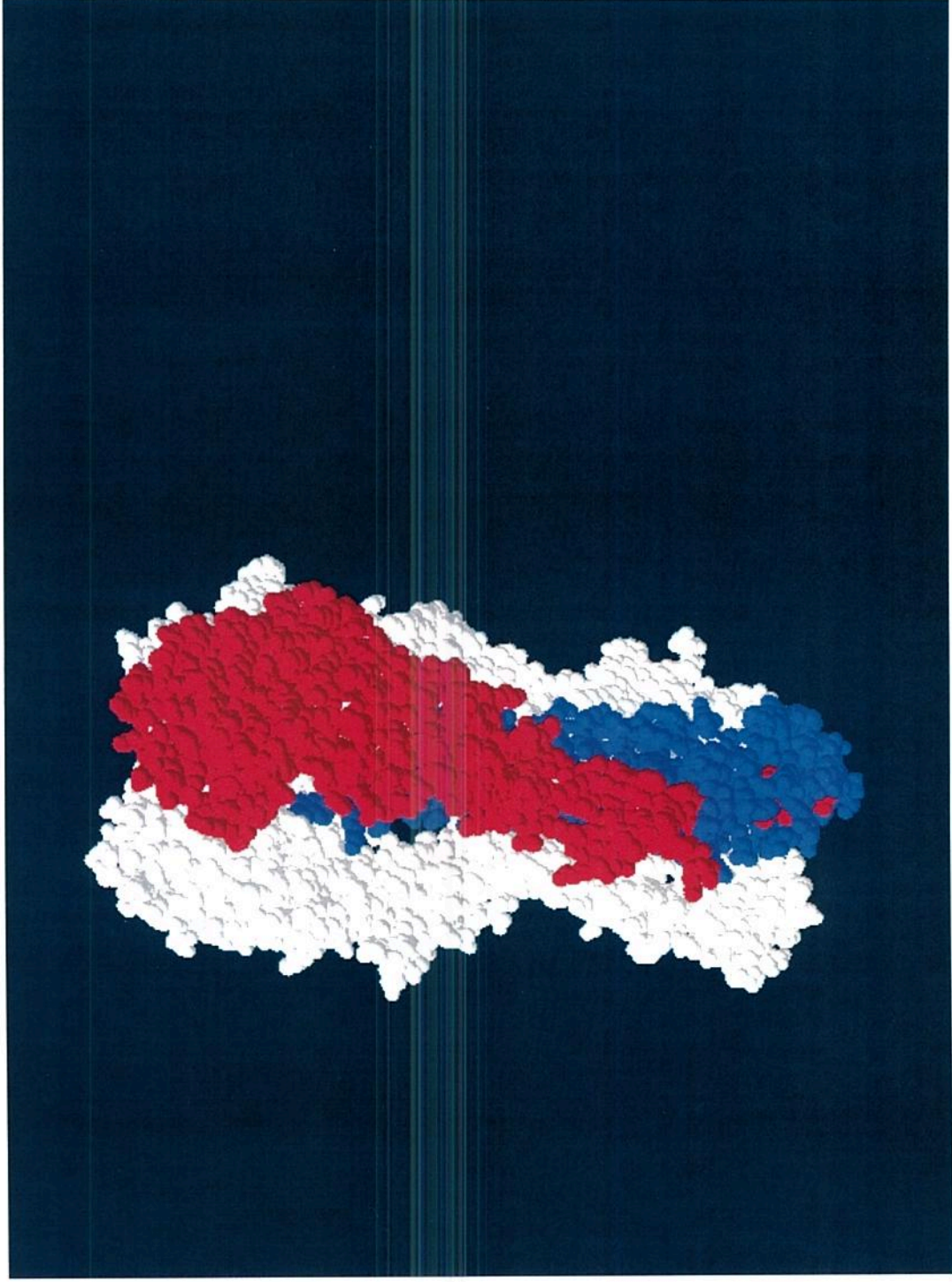
(By convention, the host of origin of human strains is not included)

Key laboratory techniques instrumental to influenza surveillance/vaccine contents

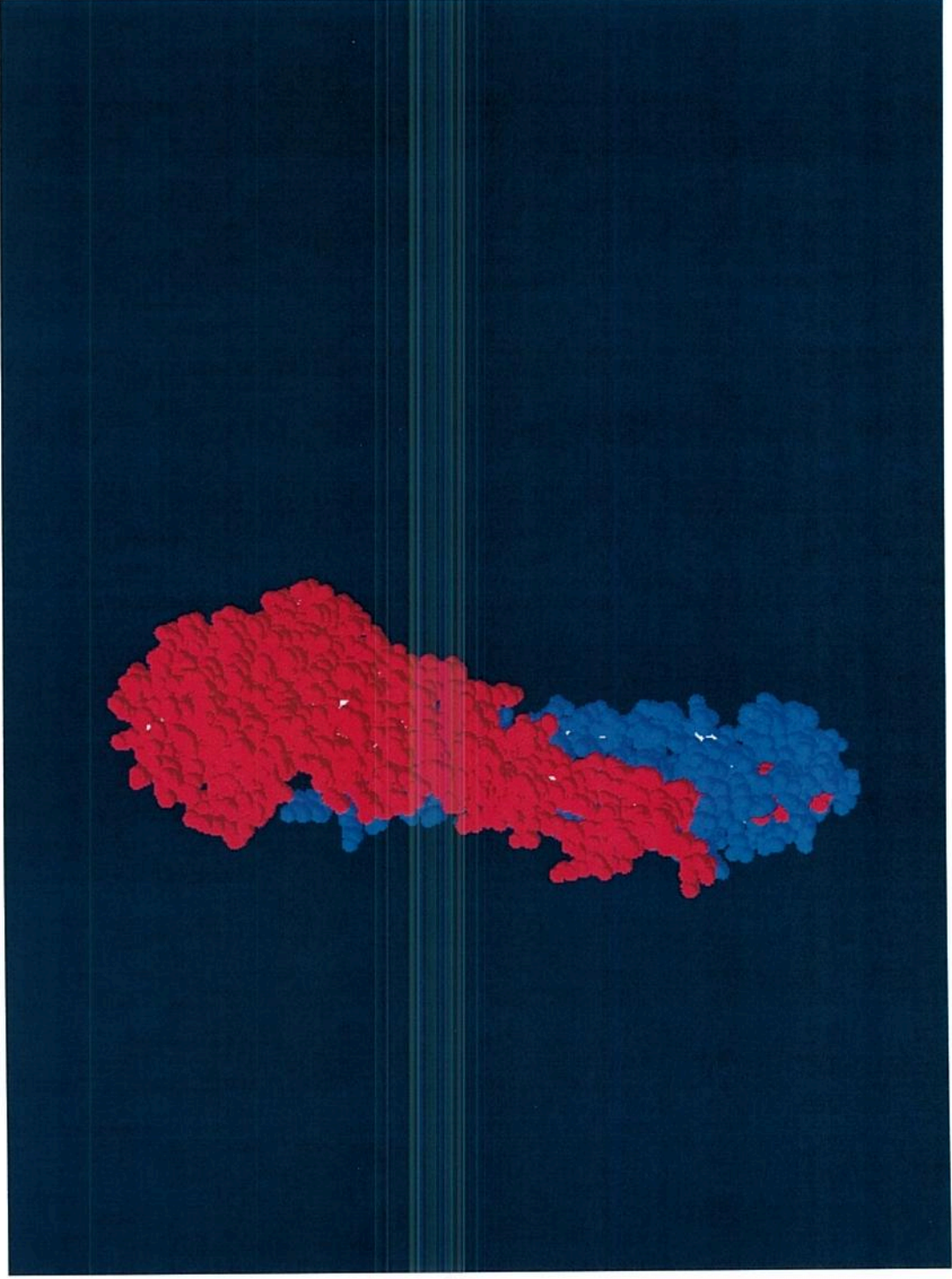
- Shot Versus Flu Mist
- How does Tamiflu work
- Sequencing HA
 - Culture, HAI, PCR, Serology



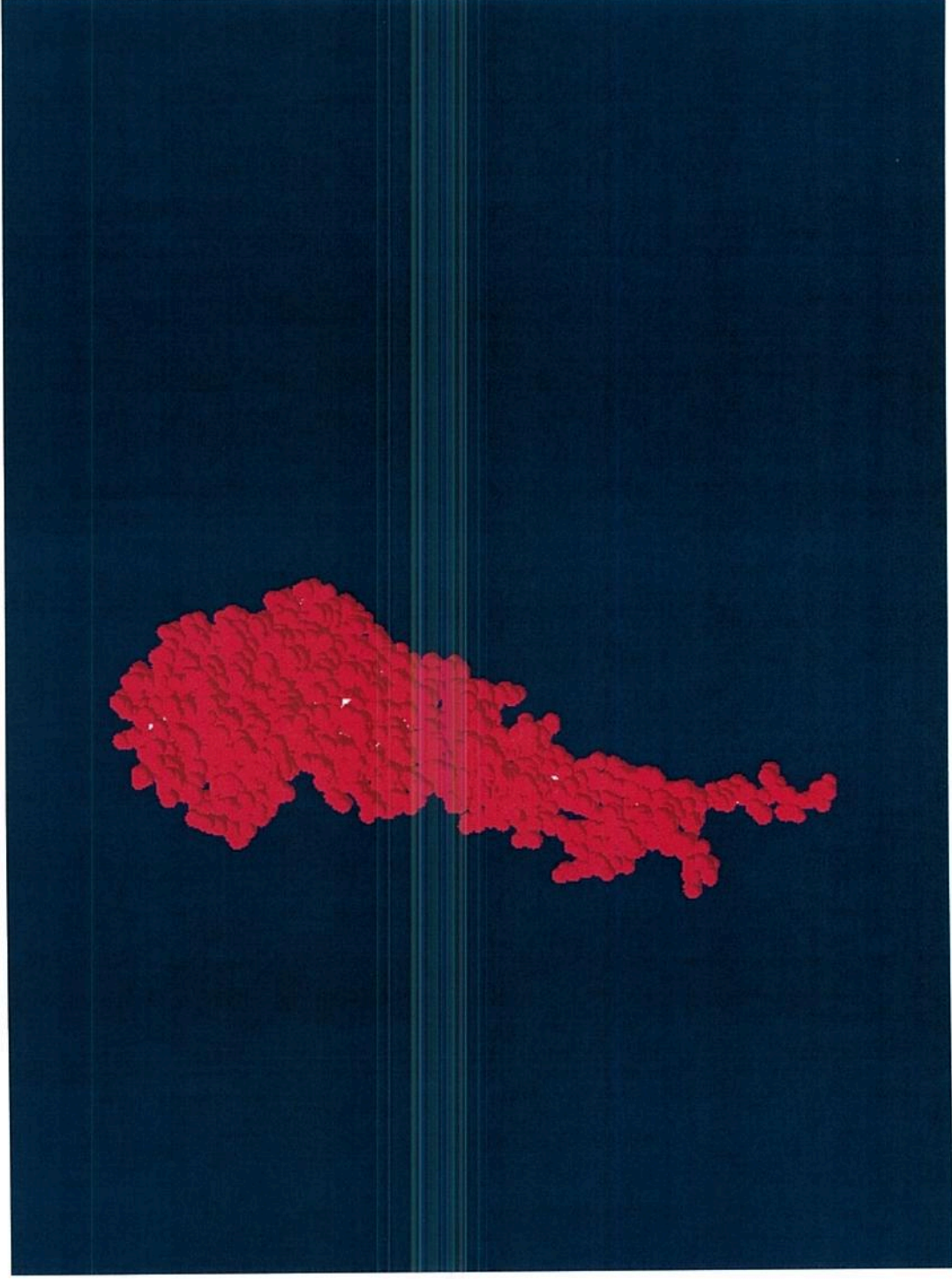
HA1 of HA



HA1 of HA

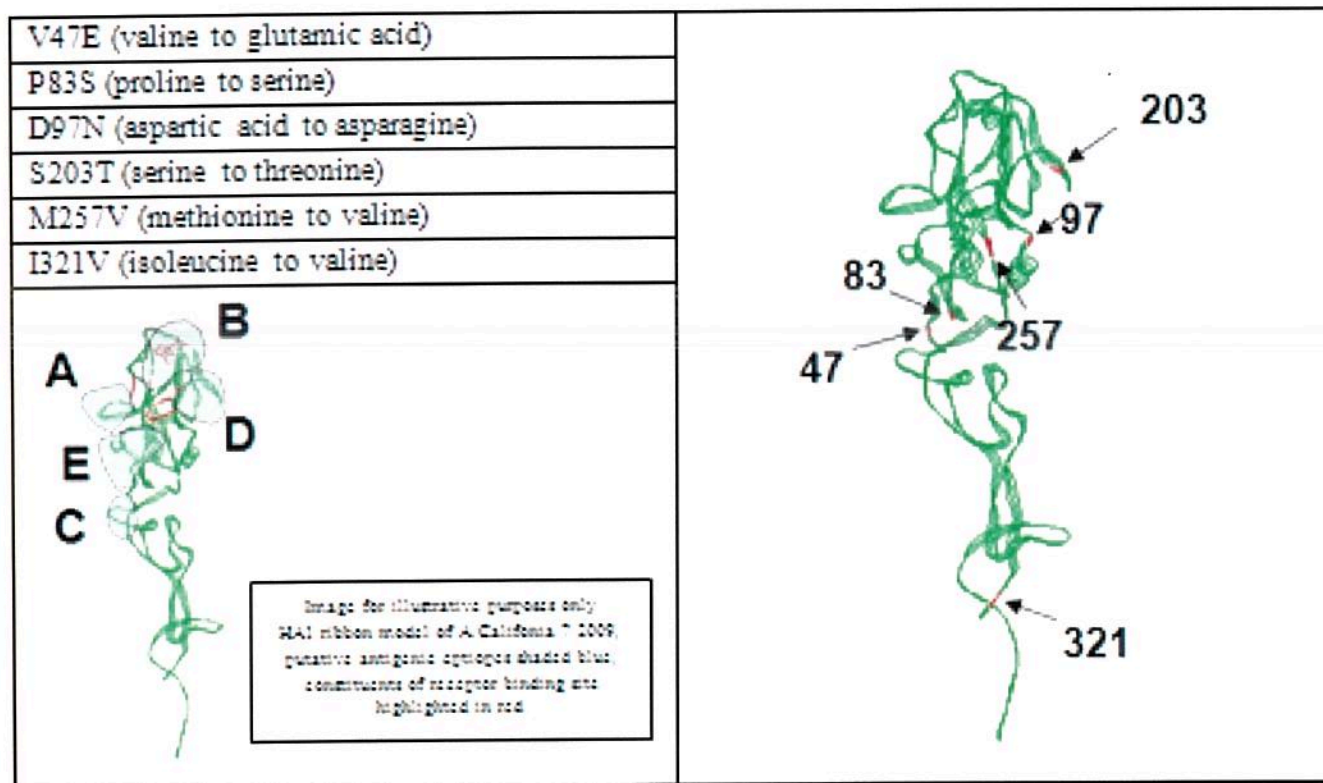


HA1 of HA



Surveillance for a new Virus

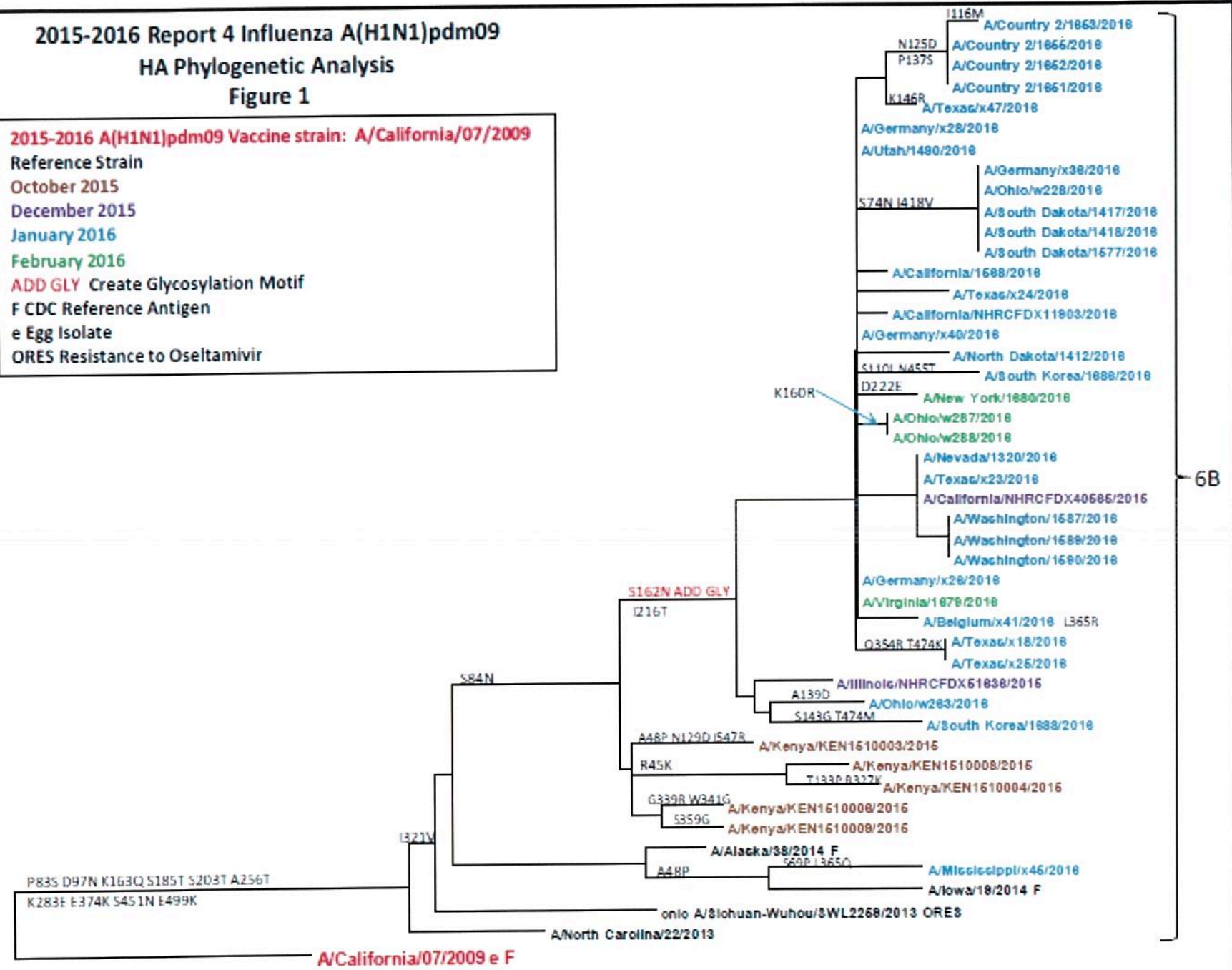
A/North Carolina/5475/2010: 98.2% overall HA1 amino acid homology



Built using first approach mode in Swiss Model. Utilized amino acids from vaccine strain and viewed in Swiss-PdbViewer with amino acids differing from vaccine strain color coded red

2015-2016 Report 4 Influenza A(H1N1)pdm09
 HA Phylogenetic Analysis
 Figure 1

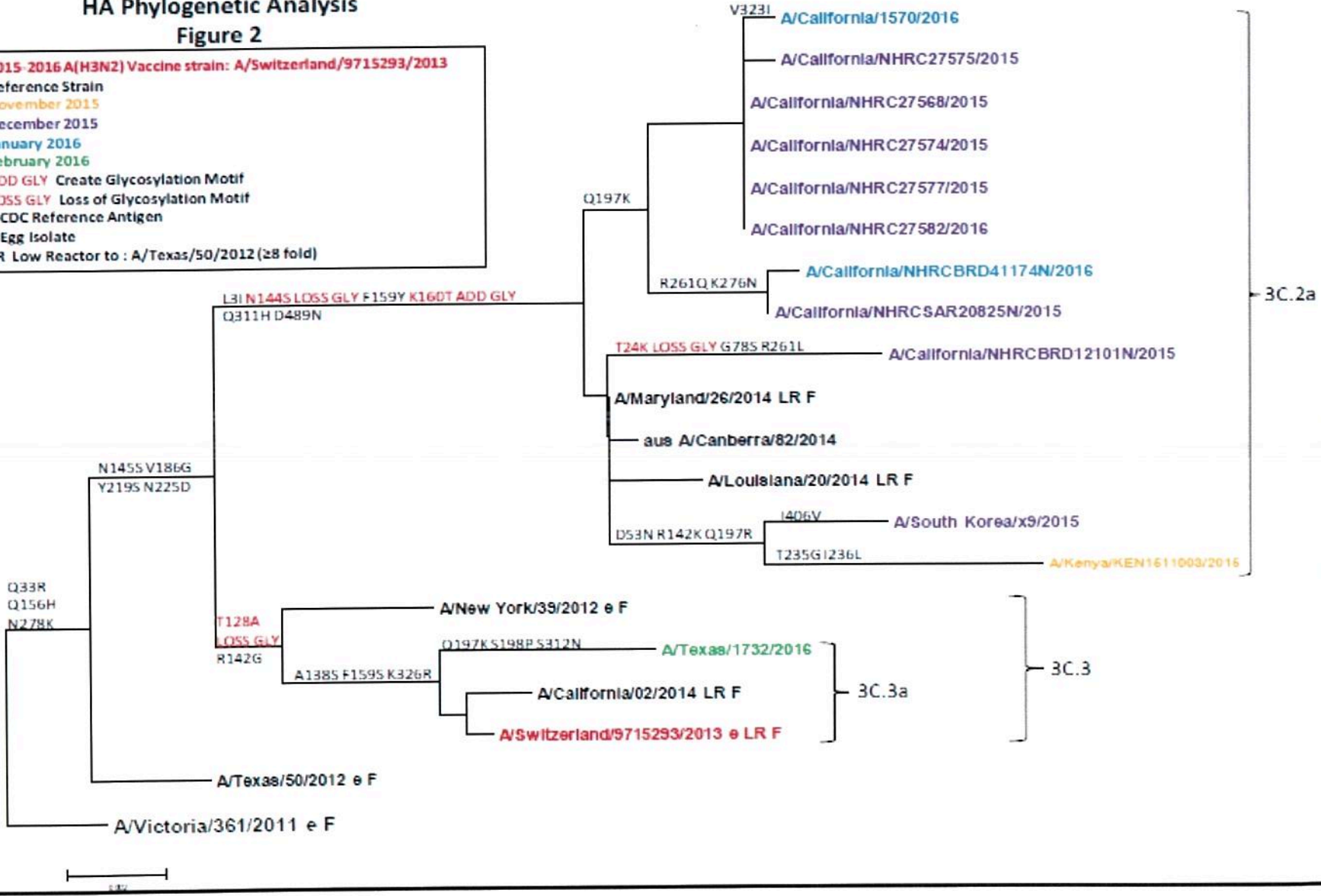
2015-2016 A(H1N1)pdm09 Vaccine strain: **A/California/07/2009**
 Reference Strain
 October 2015
 December 2015
 January 2016
 February 2016
 ADD GLY Create Glycosylation Motif
 F CDC Reference Antigen
 e Egg Isolate
 ORES Resistance to Oseltamivir

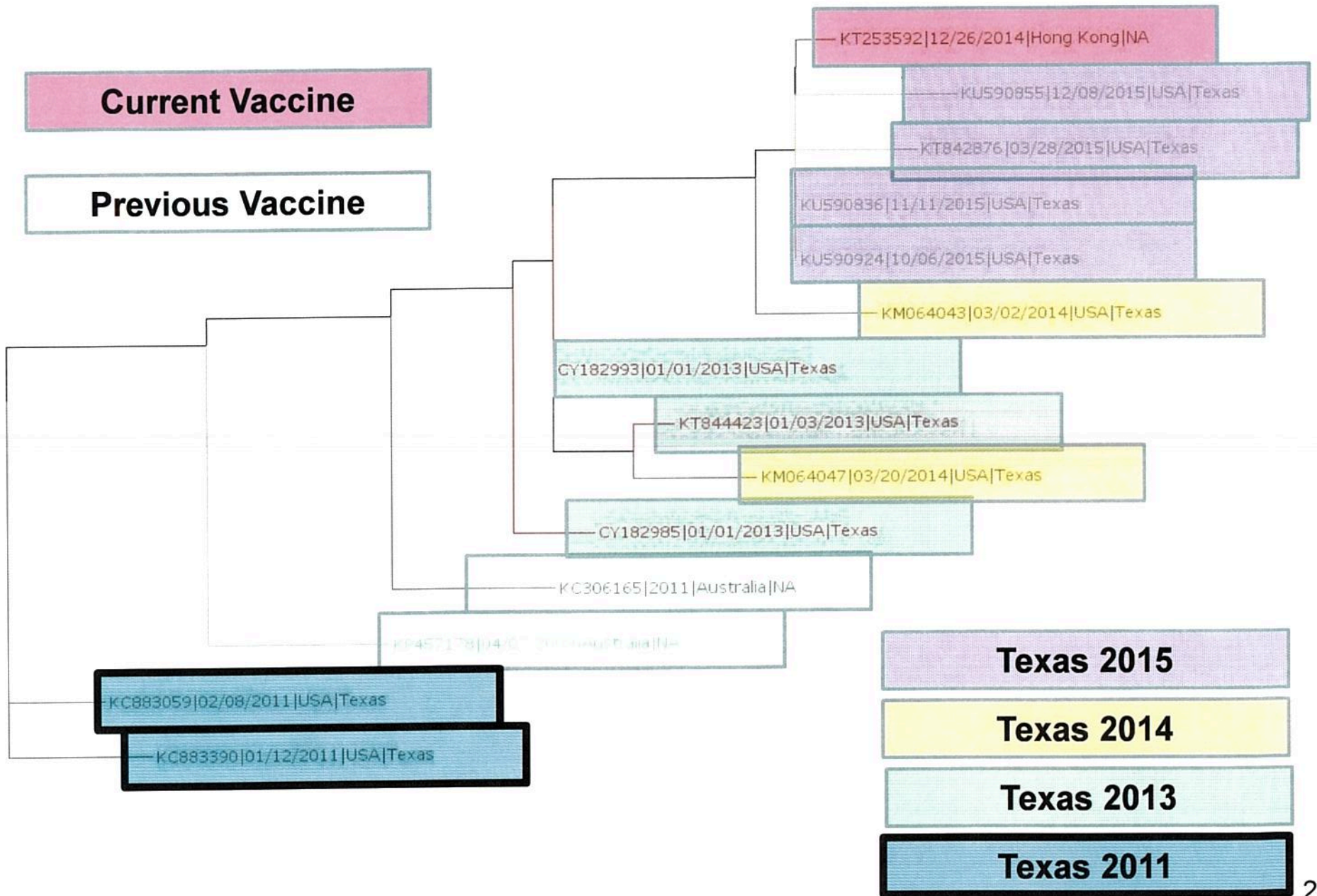


2015-2016 Report 4 Influenza A(H3N2)
HA Phylogenetic Analysis

Figure 2

2015-2016 A(H3N2) Vaccine strain: A/Switzerland/9715293/2013
 Reference Strain
 November 2015
 December 2015
 January 2016
 February 2016
 ADD GLY Create Glycosylation Motif
 LOSS GLY Loss of Glycosylation Motif
 F CDC Reference Antigen
 e Egg Isolate
 LR Low Reactor to : A/Texas/50/2012 (≥8 fold)





Questions?

References not previously listed:

- Slide 23 & 24

https://www.afhsc.mil/documents/pubs/USAFSAM_Flu_Reports/2015-2016/2016-02-20_Global%20Laboratory-based%20Influenza_Surveillance_Report.pdf

- Slide 25 & 26

Influenza Research Database (www.fludb.org), a federally funded project supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Contract No. NIH N01AI 400041