Preparing for the Fall Flu Season

Laboratory Perspective

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September 21, 2009

Objectives

- 1. Review the emergence of Novel Influenza A (H1N1) in Ontario.
- 2. Give an overview of the lab response at Ontario PHL / OAHPP.
- 3. Discuss testing guidelines.
- 4. Highlight research and surveillance projects being initiated.

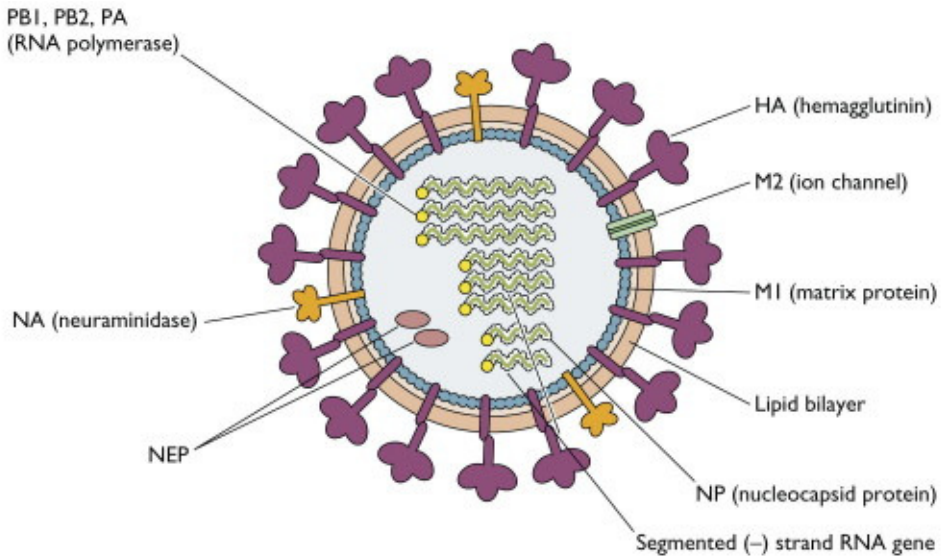
Novel H1N1 Influenza A: Background

- April 15 and 17: Novel H1N1 detected in two epidemiologically unlinked cases in U.S.
- April 20, 2009: PHAC alerted Ontario MOHLTC of SRI cases in Mexico
  - Subsequently confirmed to be novel H1N1 Influenza A
- April 28, 2009: Ontario reported 4 lab-confirmed cases.

Structure of Influenza A Viruses

8 Segments

1. **PA**
2. **PB1**
3. **PB2**
4. **HA**
5. **NP**
6. **NA**
7. **M**
8. **NS**



http://www.virology.ws/2009/04/30/structure-of-influenza-virus/

Genetic Drift and Shift

- Genetic Drift
  - **Mutations in nucleotides result in amino acid alterations.**
- Genetic Shift (Reassortment)
  - **Coinfection of cells with two different influenza A viruses that swap segments**
  - **Can theoretically result in 256 different genotypes (2^8)**

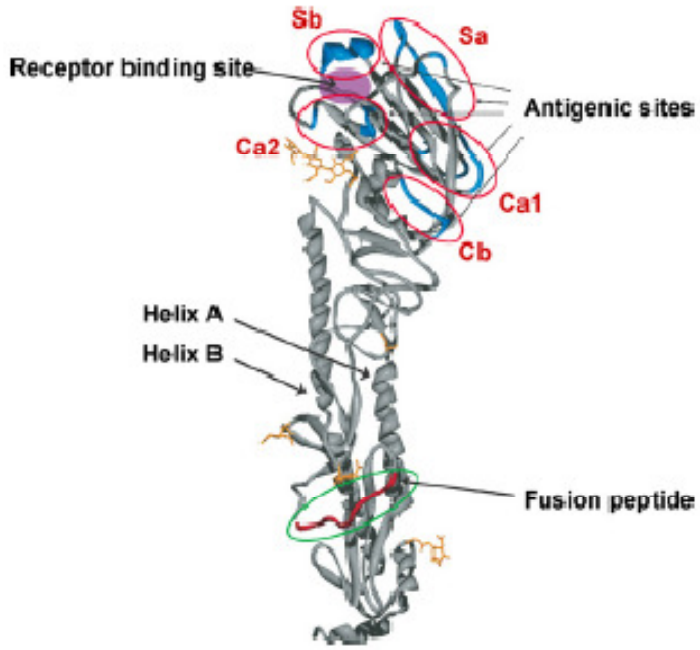
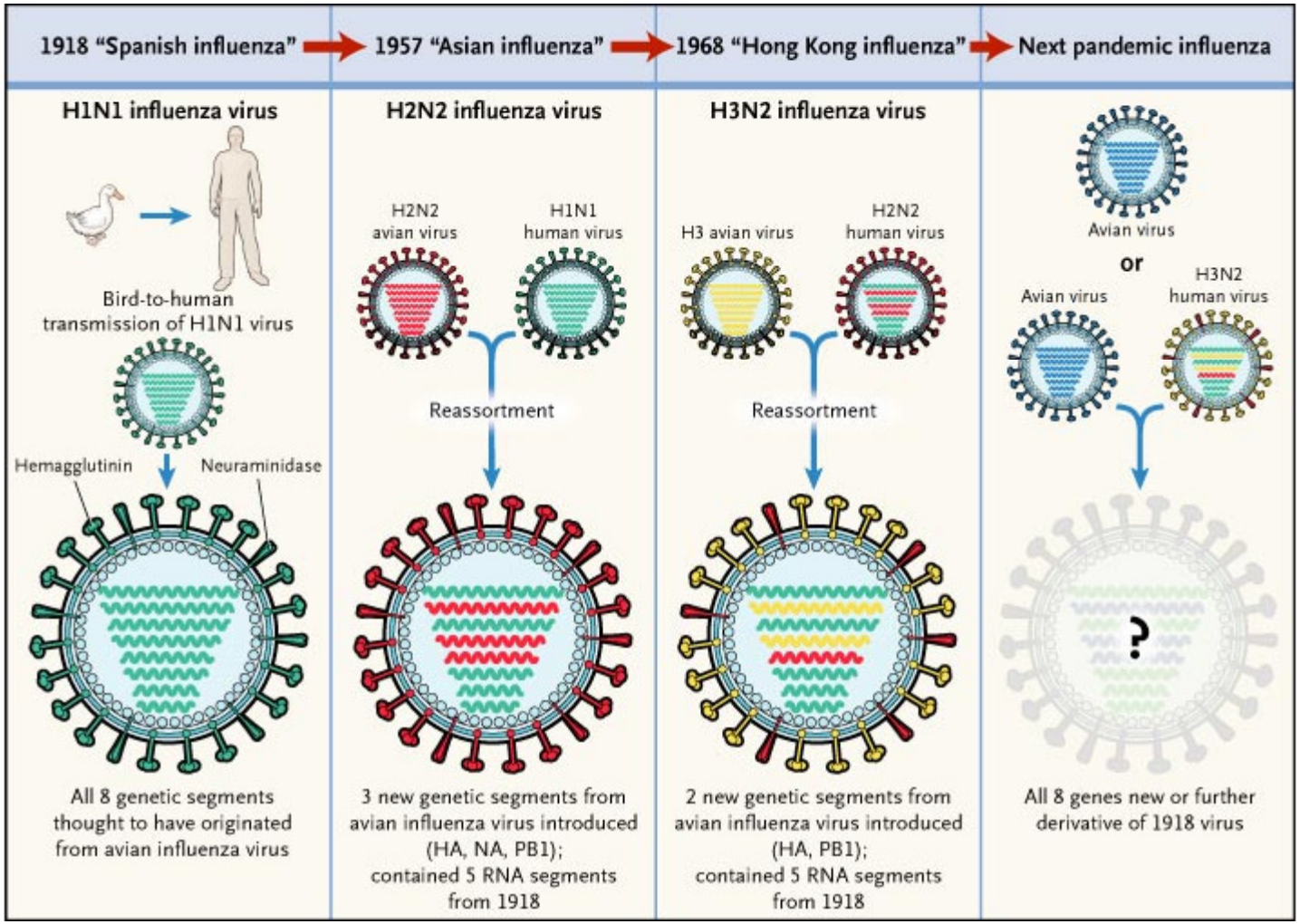


Fig. 1. Ribbon diagram of an uncleaved hemagglutinin monomer from the 1918 influenza A virus (H1N1), the causative agent of the "Spanish flu" pandemic. The head contains the sialic acid receptor-binding site, which is surrounded by the five predicted antigenic sites (Sa, Sb, Ca1, Ca2, and Cb). The stem comprises helices A and B and the fusion peptide, as shown. (Adapted from a figure, kindly provided by James Stevens and Ian Wilson, in [1].)

Fields virology. Philadelphia: LippincottWilliams &Wilkins; 2007.

Antigenic Shift



 http://content.nejm.org/content/vol353/issue21/images/large/01f1.jpeg

Novel Influenza A (H1N1): Evolution

- Triple reassortant influenza viruses from humans, pigs, and birds
  - **triple reassortant swine influenza (H1) viruses**
  - **Have circulated in pigs for >10 years.**

- Novel swine origin Influenza A (H1N1)
  - **A recent reassortant of triple reassortant swine influenza A and a Eurasian swine influenza.**

Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans

Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team*

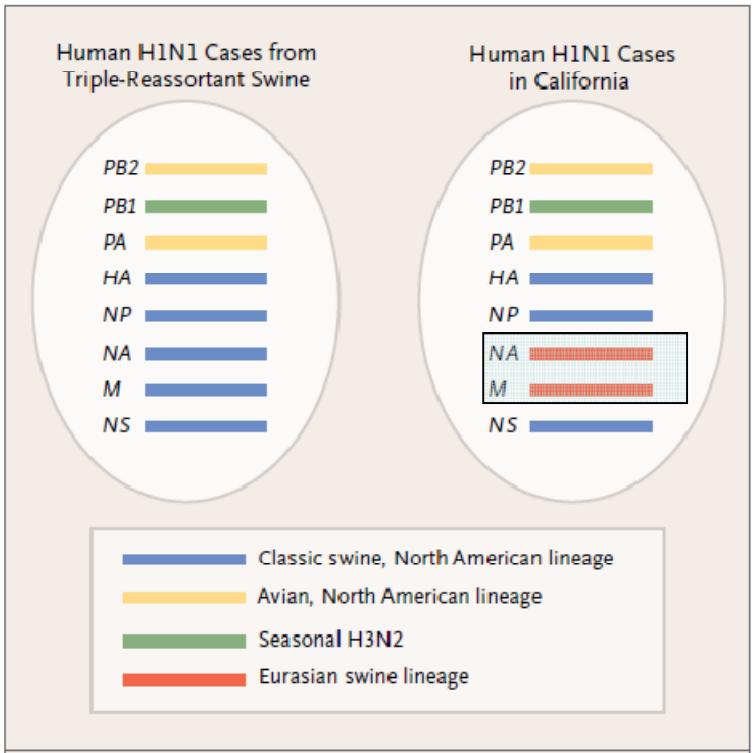


Figure 3. Comparison of H1N1 Swine Genotypes in Recent Cases in the United States.

The triple-reassortant strain was identified in specimens from patients with infection with triple-reassortant swine influenza viruses before the current epidemic of human infection with S-OIV. *HA* denotes the hemagglutinin gene, *M* the M protein gene, *NA* the neuraminidase gene, *NP* the nucleoprotein gene, *NS* the nonstructural protein gene, *PA* the polymerase PA gene, *PB1* the polymerase PB1 gene, and *PB2* the polymerase PB2 gene.

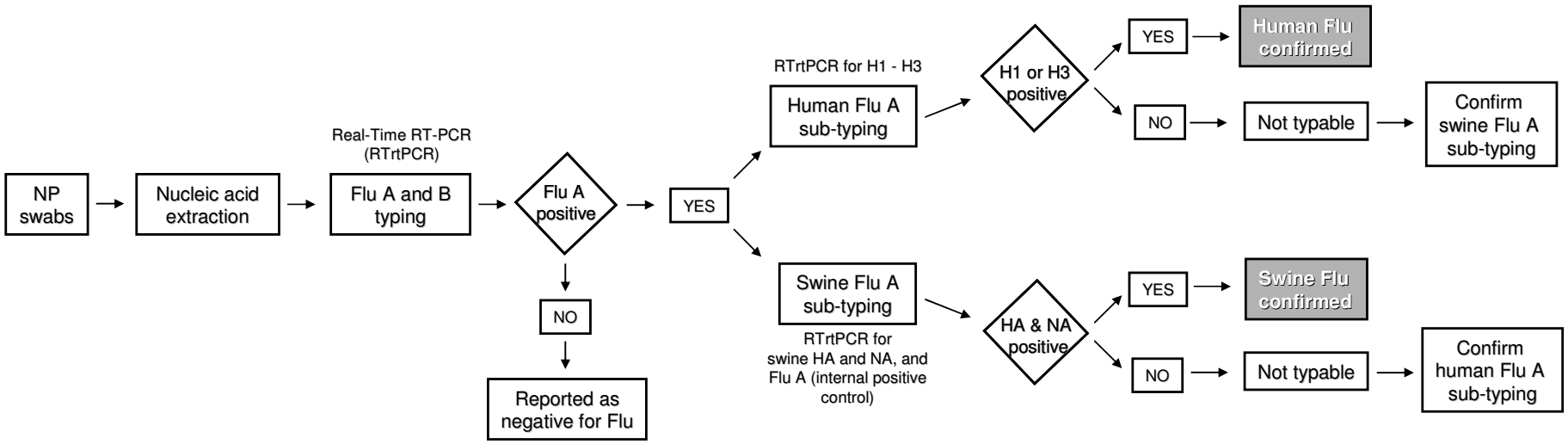
N Engl J Med 2009;361.

Initial Priorities

- Rapid implementation of a new test
- Information gathering
- Biosafety/staff education
- Surge Capacity
  - Staff redeployment
  - Equipment and supplies

Implementation of a New Test

- Establishing a new test - validation
- CDC, NML, WHO
- Design and validation of our own realtime assays to allow quicker testing
- Supporting hospitals wanting to test


NP swabs
Nucleic acid extraction
Real-Time RT-PCR (RTrtPCR)
Flu A and B typing
Flu A positive
NO
Reported as negative for Flu
YES
RTrtPCR for H1 - H3
Human Flu A sub-typing
H1 or H3 positive
YES
Human Flu confirmed
NO
Not typable
Confirm swine Flu A sub-typing
Swine Flu A sub-typing
RTrtPCR for swine HA and NA, and Flu A (internal positive control)
HA & NA positive
YES
Swine Flu confirmed
NO
Not typable
Confirm human Flu A sub-typing

Distribution Challenges

- **Testing**
- "CDC has developed a PCR diagnostic test kit to detect this novel H1N1 virus and has now distributed test kits to all states in the U.S. and the District of Columbia and Puerto Rico. **The test kits are being shipped internationally as well. This will allow states and other countries to test for this new virus**"

http://www.cdc.gov/h1n1flu/update.htm

Evaluation of in-house rtRTPCR

TABLE : Parallel sub-typing by real-time RT-PCR of influenza A positive specimens included (1) testing for human H1 and H3 and (2) human swine influenza outbreak strain requiring positive detection of both hemaglutanin (HA) and neuraminidase (NA).

Human Swine Sub-typing Result	Human Sub-typing Result		
	Unable to type*	Human Influenza A (H1)	Human Influenza A (H3)
Human Swine Flu Detected	1124		
Human Swine Flu Not Detected		21	146
Unable to type*	41		
Indeterminate◊	56		1
Total	1221	21	147

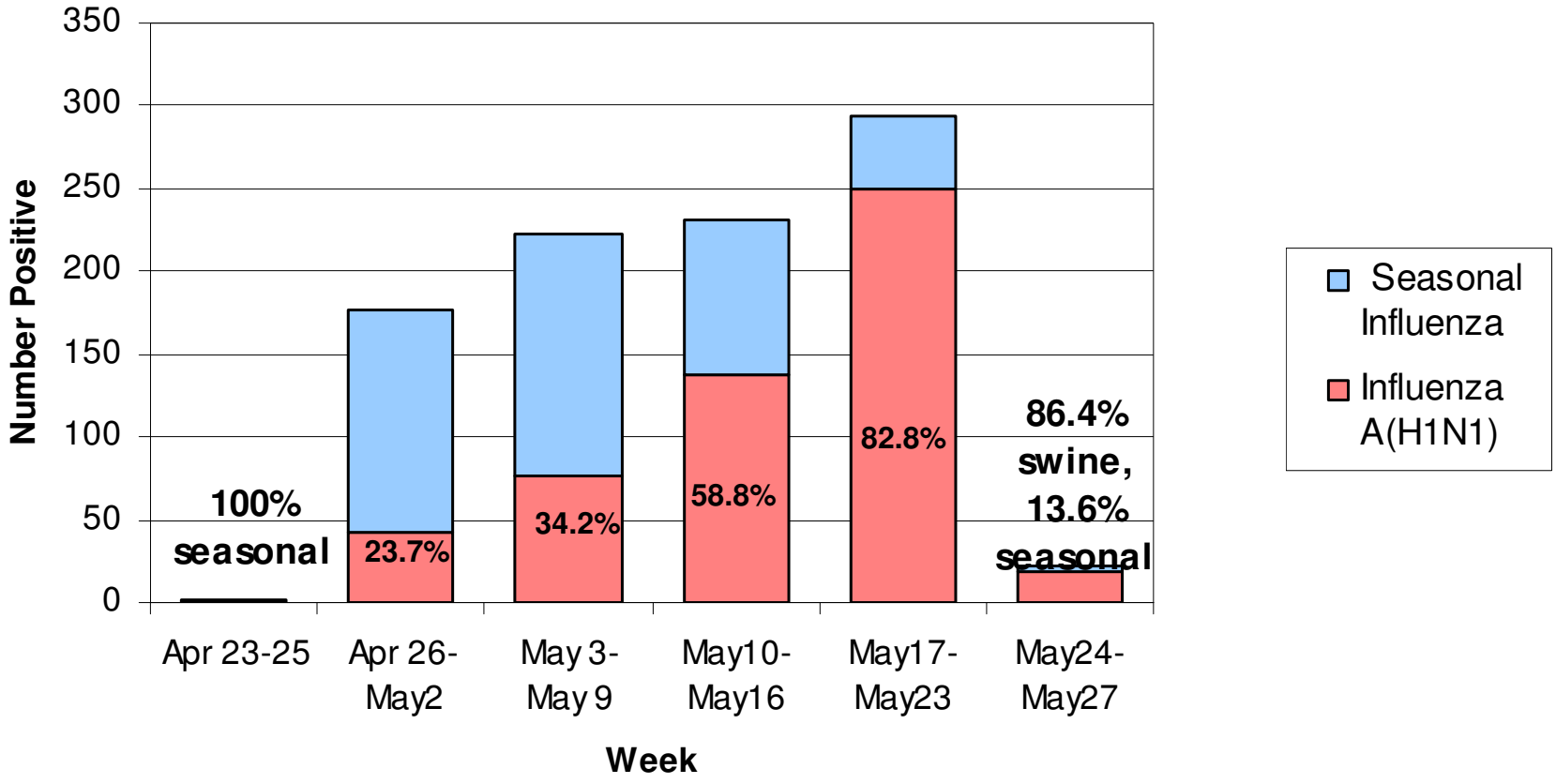
* No detection of sub-types and Influenza A cycle threshold (C_T) values > 37.

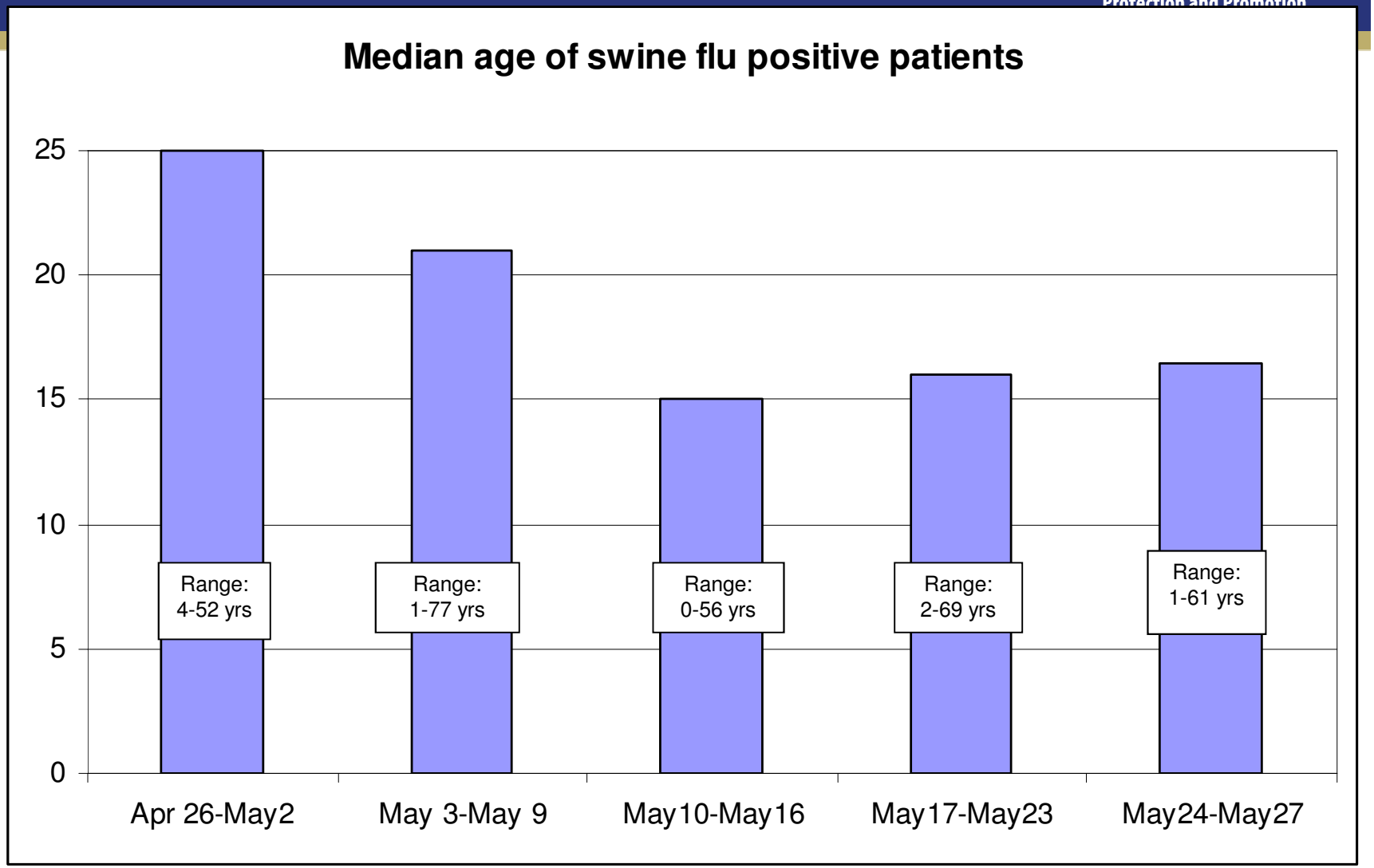
◊ Detection of only 1 human swine sub-typing target (HA or NA)

In-house rtRTPCR

- Scientific team continues to monitor circulating strains for match with PHL primers/probes
- Some mutations appearing in virus, but primers/probes remain a 100% match

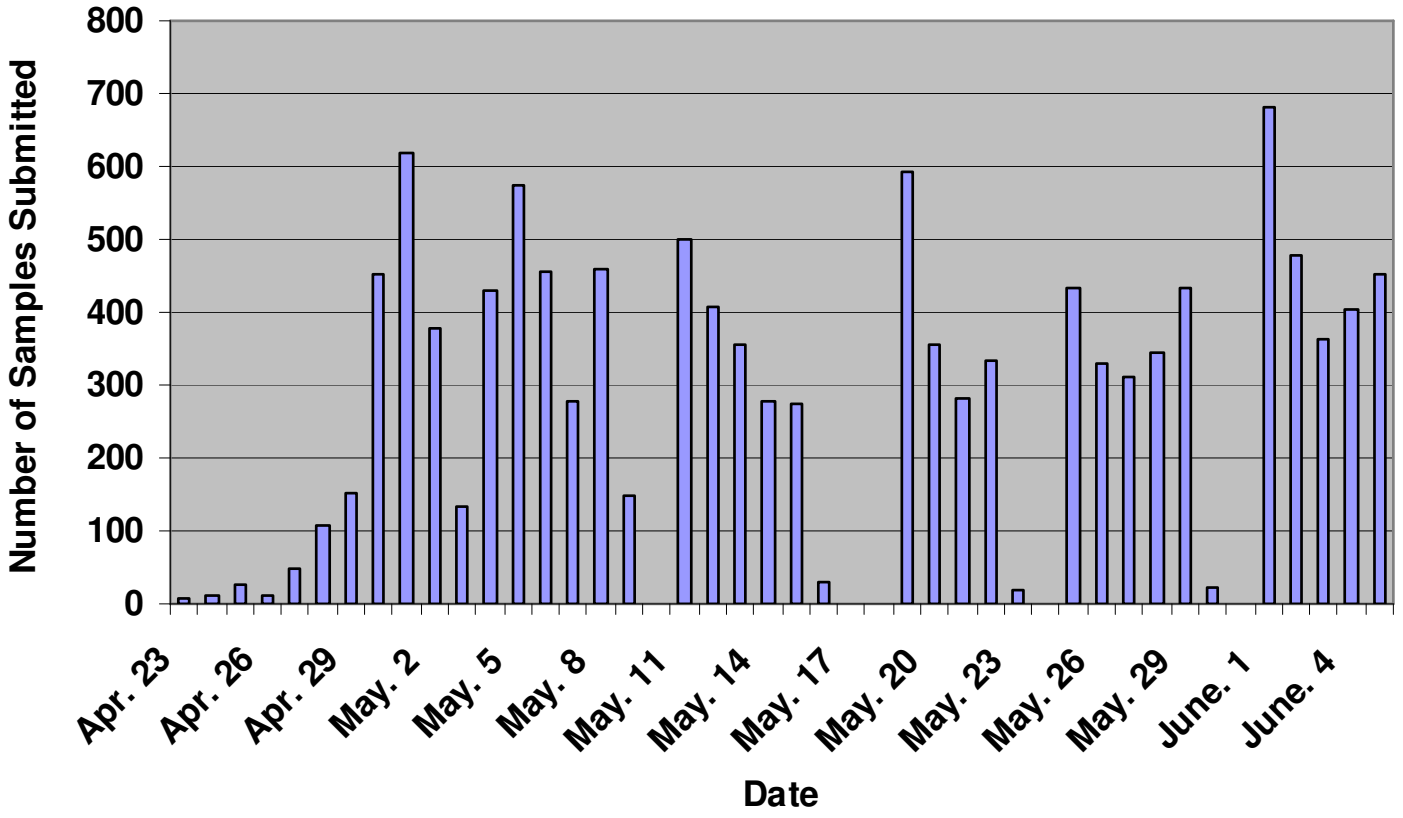
Subtyping Breakdown of Influenza A Positive Samples





Median age has dropped over time – likely due to school clusters

Number of Patients With Samples Submitted for Influenza A (H1N1) Testing



Mean 272 (313 since April 30), range 7 to 682

Important Health Notice

June 11, 2009

- On June 11, 2009, the World Health Organization (WHO) raised the pandemic alert level from Phase 5 to Phase 6.

Reminder: Direction on Laboratory Testing

Testing for novel H1N1 Influenza A is not recommended for patients with mild illness. Specimens should only be submitted for testing where lab results are required for clinical management of hospitalized cases of ILI or where patients are at high risk for complications from influenza.

As of September 16, 19223 patients with specimens submitted to OPHL

- **Flu A positive = 4176**
- Flu A Negative = 14718
- Indeterminate = 113
- Flu testing in progress = 216

- **Swine flu positive (confirmed) = 3322**
- **Human H3 = 252**
- **Human H1 = 45**
- Subtype pending = 76

Sample submissions

- 34% of specimens from persons <19yo
- 40% from persons 20-49yo
- school-aged children (5-19yo) 23% of tests, but 42% of all fluA pos.

370 hospitalizations in Ontario as of September 3

Ontario **Ontario Influenza Bulletin 2008-2009 Season** Ministry of Health & Long-Term Care Public Health Division

SURVEILLANCE WEEK 35 (August 30, 2009– September 5, 2009)

Table 5. Hospitalizations among confirmed cases of pandemic (H1N1) 2009 in Ontario, April 13 – September 3, 2009

Hospitalization Status	Ventilator and/or ICU	Not in ICU and not on ventilator	Number of cases hospitalized to date
Number Currently Hospitalized	13 (65%)	7 (35%)	**20 (100%)**
Number Hospitalized and Discharged	63 (18%)	287 (82%)	**350 (100%)**
Total Hospitalized to date	**76 (20%)**	**294 (80%)**	**370 (100%)**

Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted at 8:30 am [09/09/2009]

Ontario

Ontario Influenza Bulletin 2008-2009 Season

Ministry of Health & Long-Term Care
Public Health Division

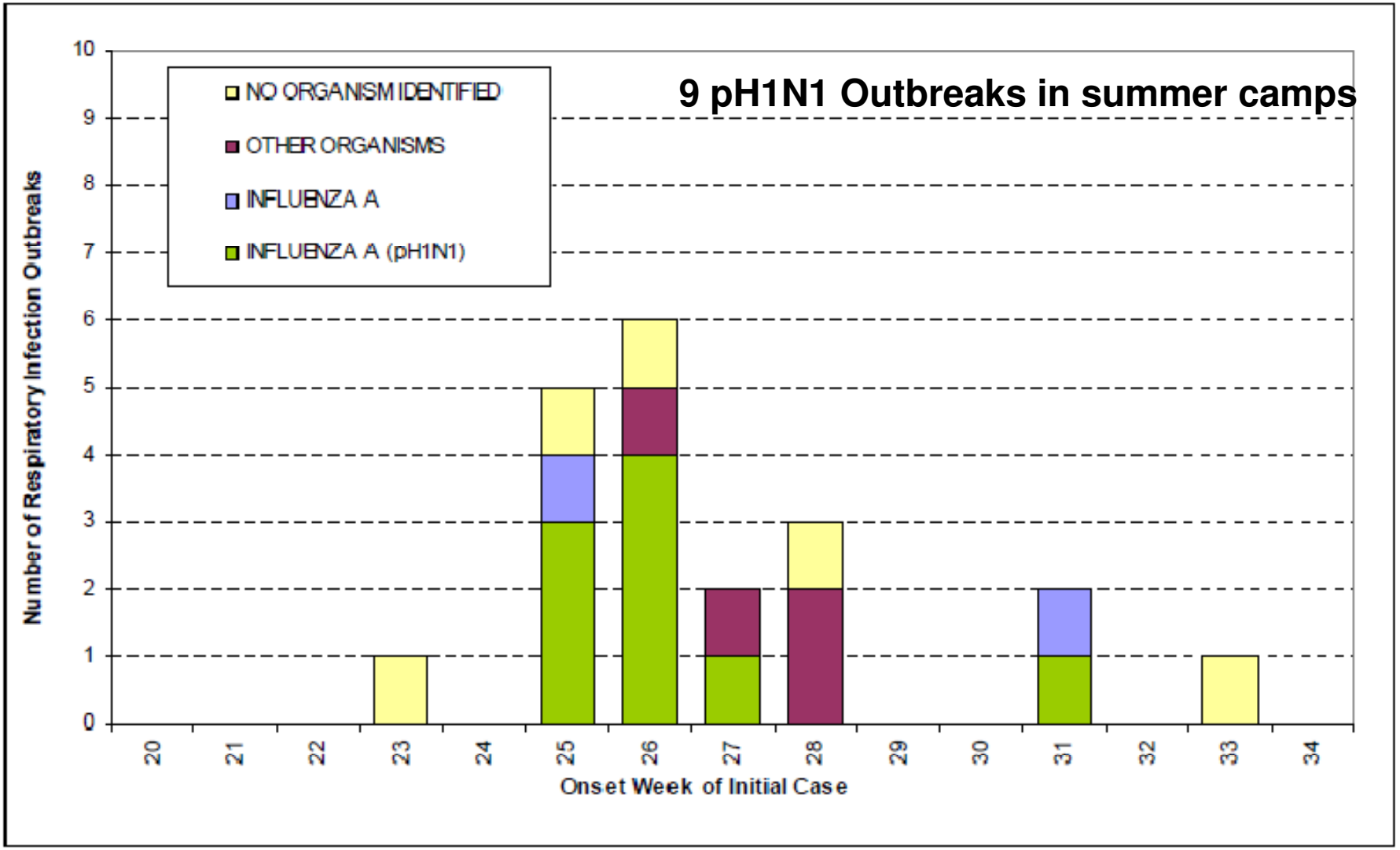
SURVEILLANCE WEEK 35 (August 30, 2009– September 5, 2009)

Table 6. Incidence of hospitalization and death due to pandemic H1N1 2009 in Ontario, April 13 – Septe 2009

Age Group	Hospitalizations	Rate/100,000	Deaths	Rate/100,000
<1	20	14.93	0	0.00
1-4	50	9.15	0	0.00
5-19	107	4.43	3	0.12
20-49	108	1.90	4	0.07
50-64	50	2.08	9	0.37
65+	35	2.03	7	0.41
TOTAL	**370**	**2.86**	**23**	**0.18**

Source (incidence): Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted at 8:30 am [09/09/2009]
Source (incidence): Ontario population projections for 2008: Ontario Ministry of Health and Long-Term Care, Public Health Planning Database (PHPDB), extracted [12/02/2009]

Figure 10: Outbreaks in Ontario summer camps by onset of illness in the first case: Total Outbreaks from June 1 – September 2, 2009 by causative organism



Source: Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System (iPHIS) database, extracted [03/09/2009]

Important Health Notice

Information for Healthcare Professionals

August 18th, 2009
Volume 6, Issue 17
Page 1 of 3

H_1N_1 UPDATE

Laboratory Testing:

Currently testing is only recommended for persons admitted to hospital and those ambulatory patients at higher risk of complications (e.g., persons with pre-existing medical conditions, pregnant, persons under the age of 2 years and over 65 years of age) with ILI.

Persons seen in emergency departments and discharged home should not be tested.

Laboratory requisitions should be clearly labelled to identify the patient as "Hospitalized" or "High-Risk" to allow appropriate triage of specimens for testing.

Current Provincial Surveillance Case Definition (as of May 5, 2009):

Confirmed
Person with Influenza-like illness[1] and

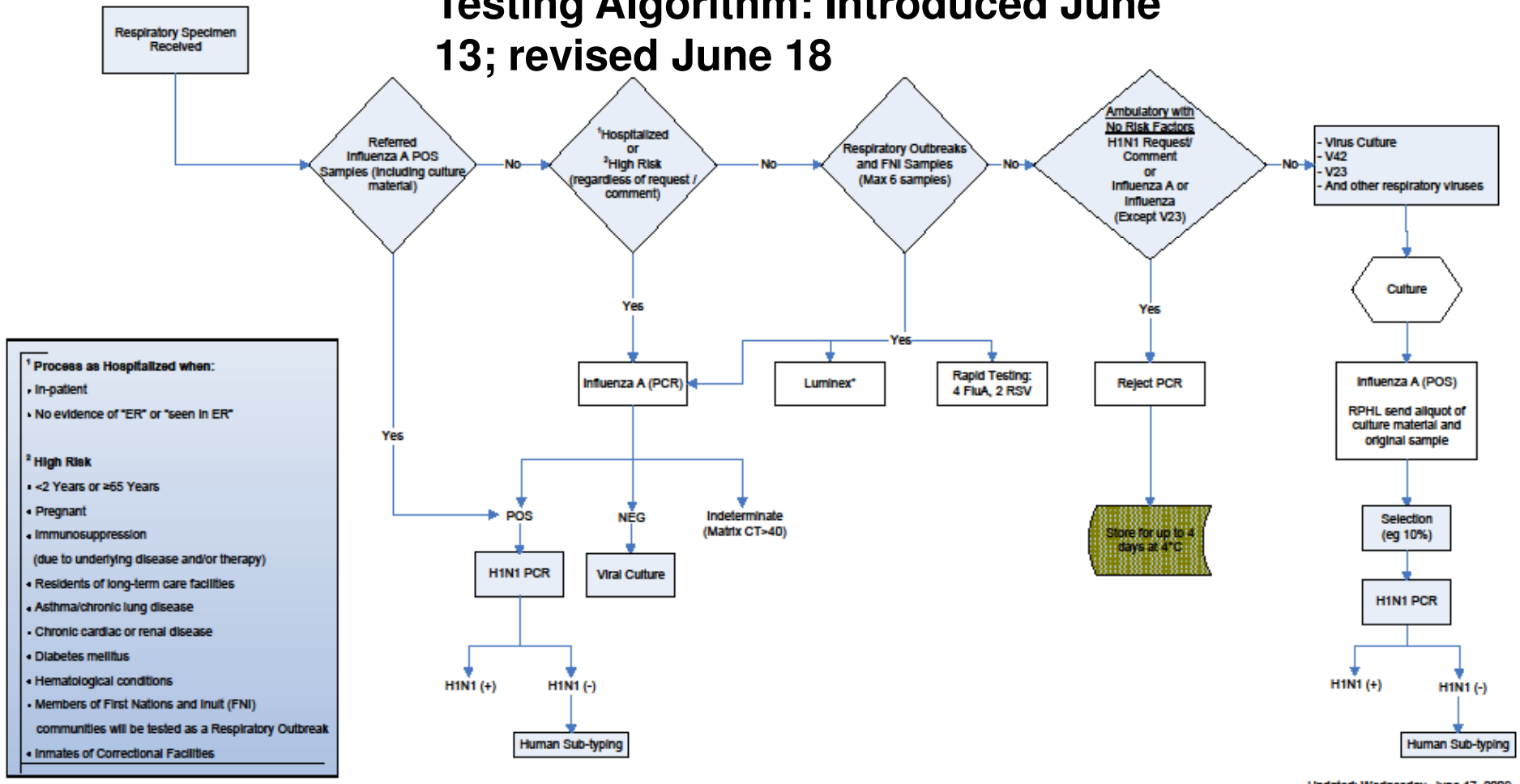
Laboratory confirmation of swine influenza A (H1N1) virus infection by one or more of the following tests:

- RT-PCR with genotyping of H1 and/or N1 swine influenza virus
- Viral culture with strain typing
- Four-fold rise in swine influenza A(H1N1) virus specific antibodies by serology testing

[1]Influenza-like illness: Acute onset of respiratory illness with fever AND one or more of the following - cough, sore throat, arthralgia, myalgia, prostration, or malaise. In cases under 5 or 65 and older fever may not be prominent.

Process for Respiratory Viral Testing

Testing Algorithm: Introduced June 13; revised June 18



*Luminex assay reports the following targets: Adenovirus, Entero/Rhino, Flu A, Flu A H1 (human), Flu A H3 (human), Flu B, Parainfluenza 1,2,3,and 4, Metapneumovirus, RSV A, RSV B.

Updated: Wednesday, June 17, 2009

Fluwatch, week ending Sept 12

- Ontario: 11/985 (1.1%) FluA tests positive;
  - **2RSV, 7PIV, 6 adenovirus.**
- Canada: 105/4270 (2.5%) FluA tests positive.

- United States: 20% of FluA tests positive

Oseltamivir Resistance

- **On June 29, 2009**, the National Influenza Center in **Denmark** reported an oseltamivir-resistant novel influenza A (H1N1) virus from an unknown date. The virus was isolated from a patient who **became ill while taking a chemoprophylaxis dose** of oseltamivir to prevent influenza infection after exposure to an ill person.

- On July 2, 2009, a person infected with an oseltamivir-resistant novel influenza A (H1N1) virus was reported from **Japan** from an illness on **May 15, 2009**. This patient also **became ill while receiving oseltamivir for chemoprophylaxis.**

- On July 3, The **Hong Kong** reported a resistant virus isolated from a 16 year-old girl who had a **fever upon arrival at the Hong Kong International airport** on **June 11, 2009.**

ONTA

Surveillance for Pediatric Deaths Associated with 2009 Pandemic Influenza A (H1N1) Virus Infection — United States, April–August 2009

Protection and Promotion

MMWR

September 4, 2009 / Vol. 58 / No. 34

- Of 36 children that have died in the US, 7 (19%) were less than 5 years old.
- 24 (67%) had one or more of the high-risk medical conditions.
- Among 23 children with culture or pathology results reported, laboratory-confirmed bacterial coinfections were identified in 10 (43%)
- "Clinicians also should be aware of the potential for severe bacterial coinfections among children diagnosed with influenza and treat accordingly"

Evaluation of Rapid Influenza Diagnostic Tests for Detection of Novel Influenza A (H1N1) Virus – United States, 2009

MMWR

August 7, 2009 / Vol. 58 / No. 30

- Sensitivity of RIDTs 40-69%; as low as 10% in some studies.

- "Treatment should not await laboratory confirmation because laboratory testing can sometimes delay treatment and because a negative rapid test does not rule out influenza."

Updated Interim Recommendations for the Use of Antiviral Medications in the Treatment and Prevention of Influenza for the 2009-2010 Season

September 8, 2009 2:00 PM ET;

http://www.cdc.gov/h1n1flu/recommendations.htm

Research at OPHL/OAHPP

- Many initiatives underway
  - Evaluation of diagnostic tests
  - Monitoring of genetic mutations over time/passage/within clusters.
  - Seroepidemiology studies
  - Collaboration looking at monoclonal antibody therapy
  - Point of care test development

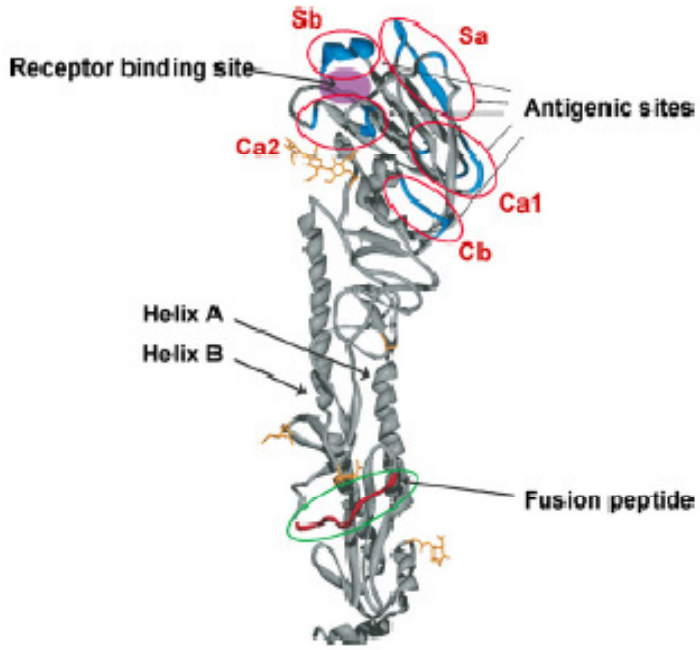


Fig. 1. Ribbon diagram of an uncleaved hemagglutinin monomer from the 1918 influenza A virus (H1N1), the causative agent of the "Spanish flu" pandemic. The head contains the sialic acid receptor-binding site, which is surrounded by the five predicted antigenic sites (Sa, Sb, Ca1, Ca2, and Cb). The stem comprises helices A and B and the fusion peptide, as shown. (Adapted from a figure, kindly provided by James Stevens and Ian Wilson, in [1].)

Fields virology. Philadelphia: LippincottWilliams &Wilkins; 2007.

Seroepidemiology

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus

Kathy Hancock, Ph.D., Vic Veguilla, M.P.H., Xiuhua Lu, M.D., Weimin Zhong, Ph.D., Eboneé N. Butler, M.P.H., Hong Sun, M.D., Feng Liu, M.D., Ph.D., Libo Dong, M.D., Ph.D., Joshua R. DeVos, M.P.H., Paul M. Gargiullo, Ph.D., T. Lynnette Brammer, M.P.H., Nancy J. Cox, Ph.D., Terrence M. Tumpey, Ph.D., and Jacqueline M. Katz, Ph.D.

Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus

- Microneutralization assay.
- Measured cross-reactive antibodies to pandemic H1N1 virus (2009 H1N1) in stored serum samples
- Cross-reactive antibodies to pH1N1 in
  - **4 of 107 (4%) persons born after 1980.**
  - **39 of 115 persons (34%) born before 1950.**

N Engl J Med 2009;361.

doi:10.1038/nature08260 nature

LETTERS

In vitro and *in vivo* characterization of new swine-origin H1N1 influenza viruses

Yasushi Itoh[1], Kyoko Shinya[2], Maki Kiso[3], Tokiko Watanabe[4], Yoshihiro Sakoda[5], Masato Hatta[4], Yukiko Muramoto[6], Daisuke Tamura[3], Yuko Sakai-Tagawa[3], Takeshi Noda[7], Saori Sakabe[3], Masaki Imai[4], Yasuko Hatta[4], Shinji Watanabe[4], Chengjun Li[4], Shinya Yamada[3], Ken Fujii[3], Shin Murakami[3], Hirotaka Imai[3], Satoshi Kakugawa[3], Mutsumi Ito[3], Ryo Takano[3], Kiyoko Iwatsuki-Horimoto[3], Masayuki Shimojima[3], Taisuke Horimoto[3], Hideo Goto[3], Kei Takahashi[3], Akiko Makino[2], Hiroshito Ishigaki[1], Misako Nakayama[1], Masatoshi Okamatsu[5], Kazuo Takahashi[8], David Warshauer[9], Peter A. Shult[9], Reiko Saito[10], Hiroshi Suzuki[10], Yousuke Furuta[11], Makoto Yamashita[12], Keiko Mitamura[13], Kunio Nakano[13], Morio Nakamura[13], Rebecca Brockman-Schneider[14], Hiroshi Mitamura[15], Masahiko Yamazaki[16], Norio Sugaya[17], M. Suresh[4], Makoto Ozawa[4,7], Gabriele Neumann[4], James Gern[14], Hiroshi Kida[5], Kazumasa Ogasawara[1] & Yoshihiro Kawaoka[2,3,4,6,7,18]

http://www.nature.com/nature/journal/vnfv/ncurrent/pdf/nature08260.pdf

In vitro and *in vivo* characterization of new swine-origin H1N1 influenza viruses

- No appreciable neutralizing antibodies against pandemic H1N1 were found for individuals born after 1920.
- Many of those born before 1918 had high neutralizing antibody titres.
- Pandemic (H1N1) 2009 isolates replicate more efficiently than human H1N1 in lungs of mice, ferrets and non-human primates.

http://www.nature.com/nature/journal/vnfv/ncurrent/pdf/nature08260.pdf

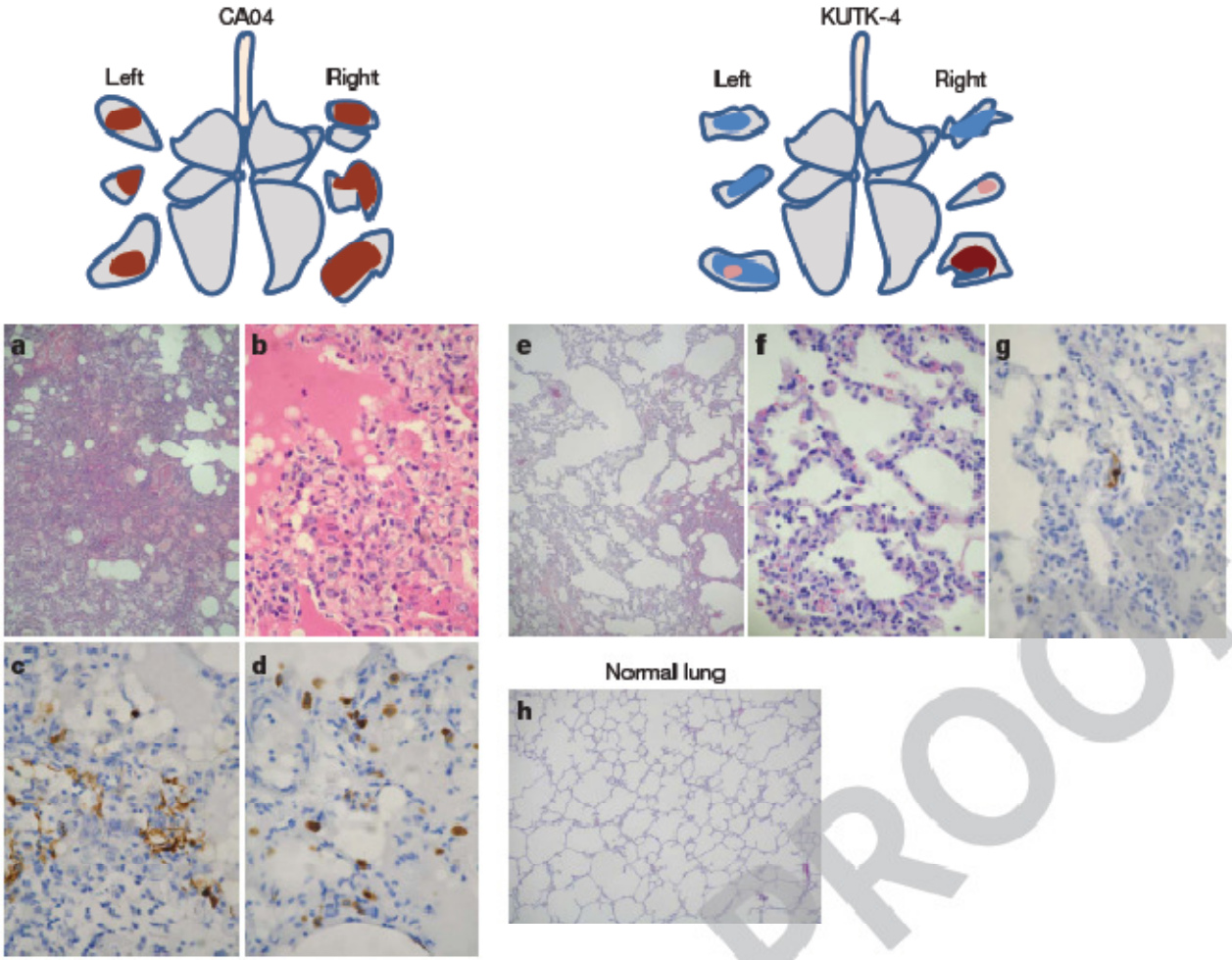
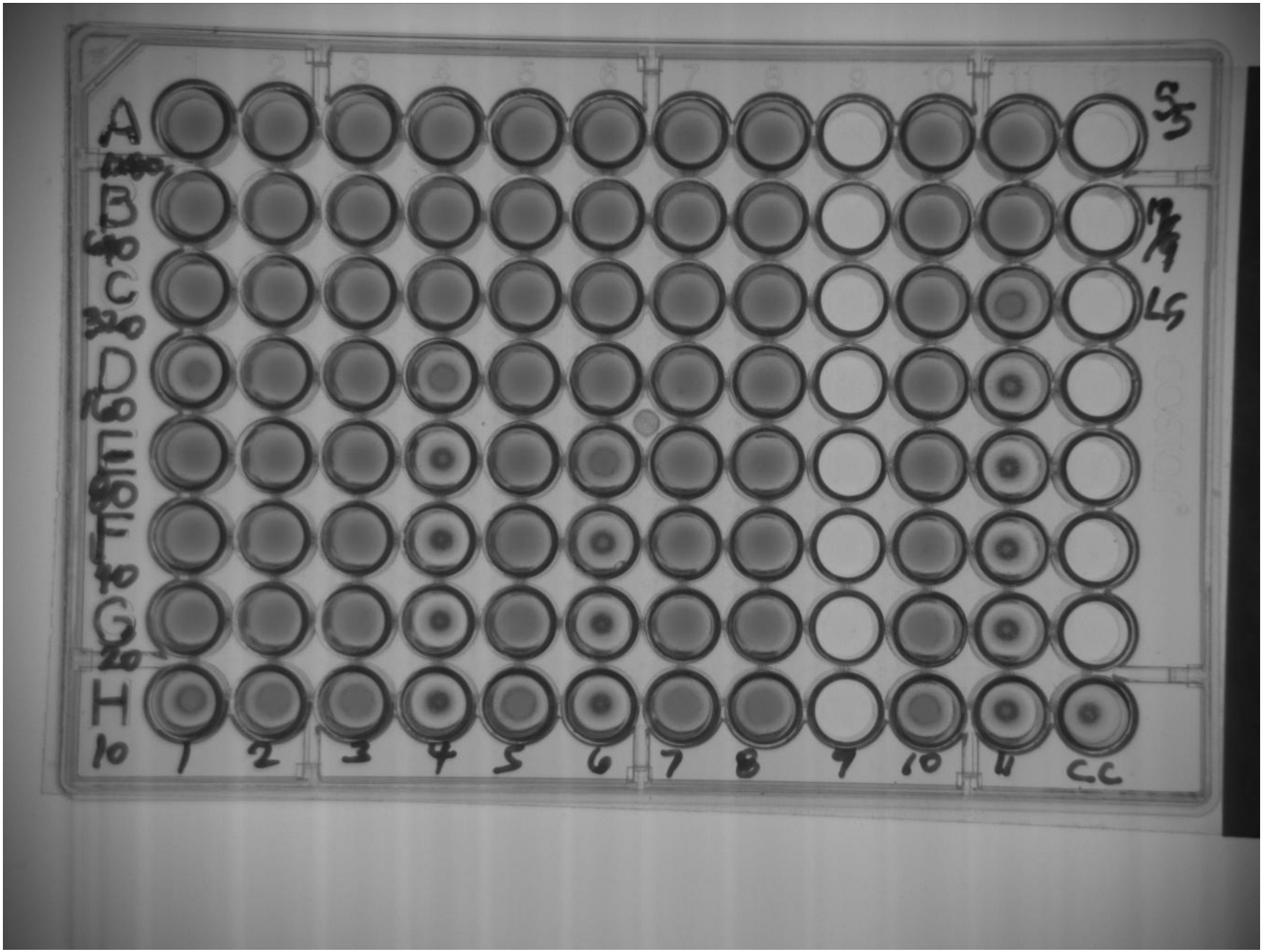


Figure 1 | Pathological examination of the lungs of infected cynomolgus macaques. **a–h**, Representative pathological images of CA04-infected (macaque no. 1, **a–d**), KUTK-4-infected (macaque no. 7, **e–g**) and mock-infected (**h**) lungs on day 3 after infection. One or two sections per lung lobe were examined. Representative findings are shown to depict the distribution of lesions in the sections (shown as cross-sections placed next to illustrations of each lung lobe), with or without viral antigen, as follows: brown, severe lung lesion containing moderate to many viral-antigen-positive cells; pink, mild lung lesions containing a few viral-antigen-positive cells; blue, lung lesions with alveolar wall thickening, with remaining air spaces unaffected. Original magnification: **a**, **e**, **h**, ×40; **b–d**, **f**, **g**, ×400.

***In vitro* and *in vivo* characterization of new swine-origin H1N1 influenza viruses**

http://www.nature.com/nature/journal/vnfv/ncurrent/pdf/nature08260.pdf

Hemagglutination Inhibition Assay



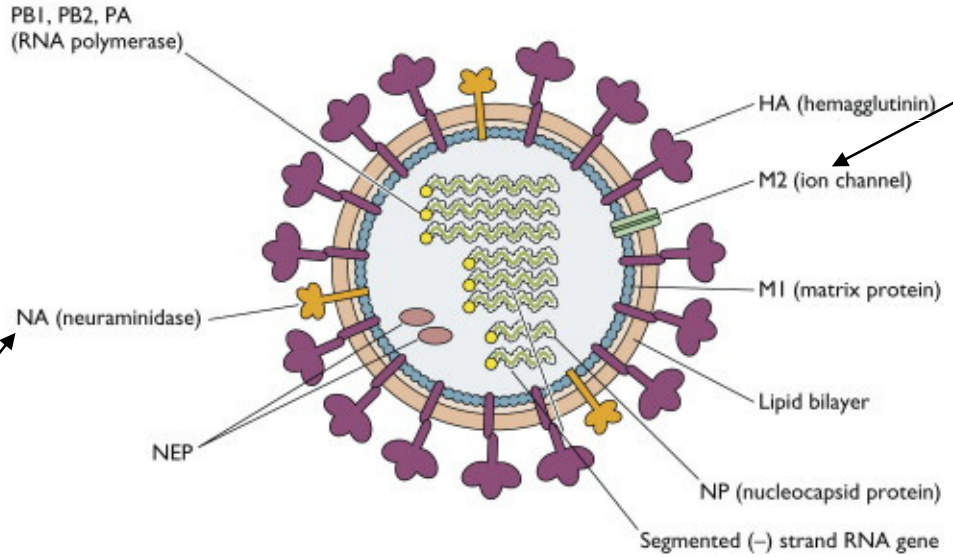
Respiratory infection outbreaks from institutions in Ontario, April 20 to June 12, 2009

- 112 outbreaks reported in Ontario.
- Only 2 of 83 outbreaks tested by molecular methods were associated with the pandemic strain.
  - One LTCF, one hospital outbreak
  - Mean age 82yrs; median 85yrs.

- In the same time period, Influenza A was detected in 2,966 (25.5%), and novel influenza A (H1N1) in 2,203 (19%) of 11,612 persons tested at PHL.
  - Mean age 21.5yrs, median 16yrs

Resistance Testing

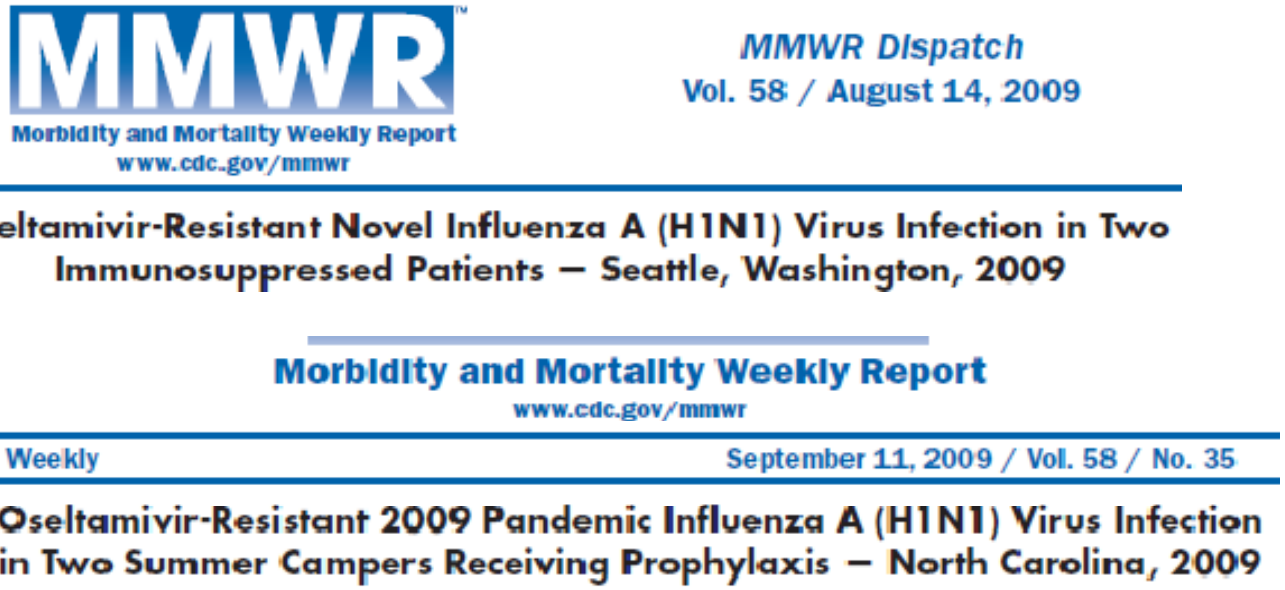
Amantadanes



Neuraminidase Inhibitors

Antiviral Resistance Testing

- Novel H1N1 are almost all oseltamivir susceptible, amantadane resistant.



MMWR
Morbidity and Mortality Weekly Report
www.cdc.gov/mmwr

MMWR Dispatch
Vol. 58 / August 14, 2009

Oseltamivir-Resistant Novel Influenza A (H1N1) Virus Infection in Two Immunosuppressed Patients — Seattle, Washington, 2009

Morbidity and Mortality Weekly Report
www.cdc.gov/mmwr

Weekly — September 11, 2009 / Vol. 58 / No. 35

Oseltamivir-Resistant 2009 Pandemic Influenza A (H1N1) Virus Infection in Two Summer Campers Receiving Prophylaxis — North Carolina, 2009

Potential for antiviral resistance development on prophylaxis

- "Use of antiviral medications for postexposure **chemoprophylaxis should be reserved for persons at higher risk for influenza-related complications** who have had contact with someone likely to have been infected with influenza".

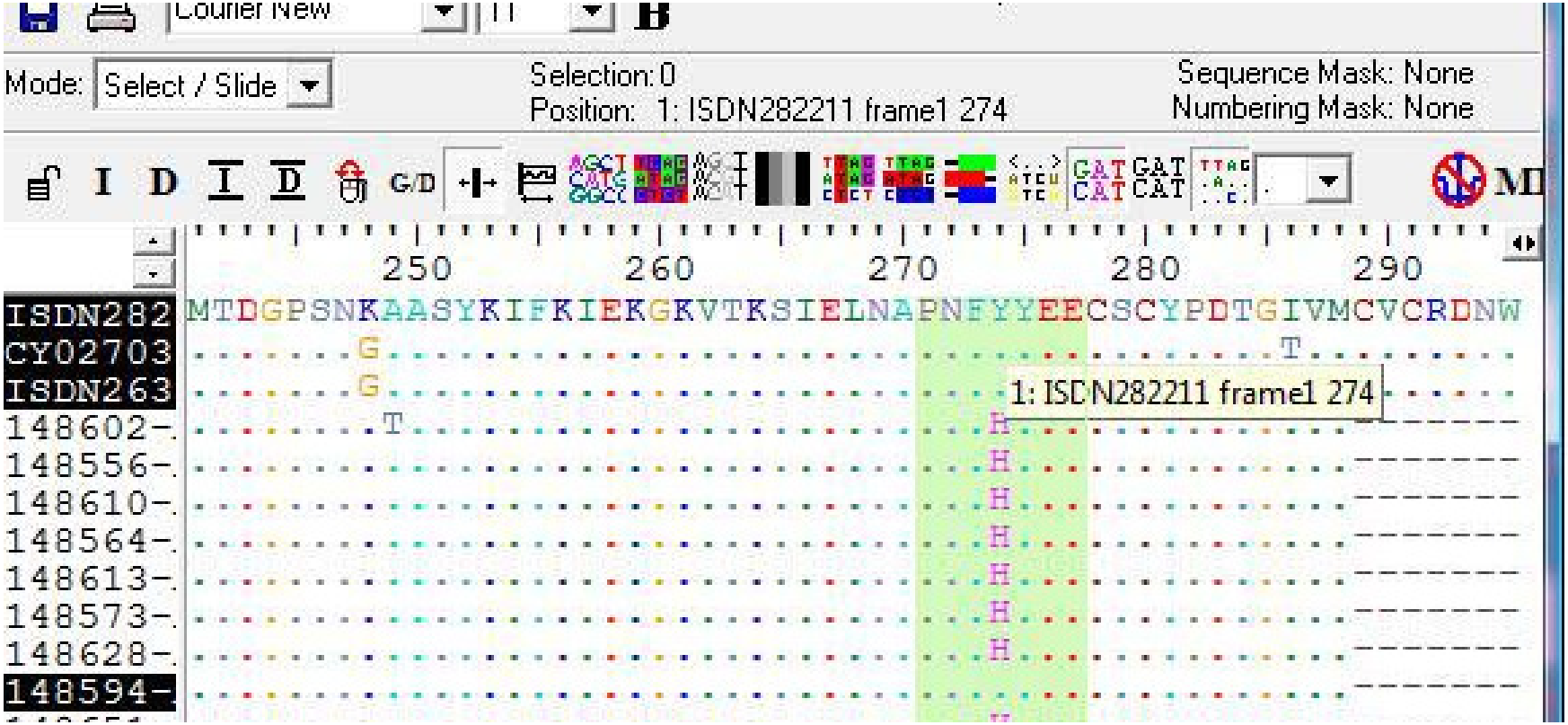
- Updated Interim Recommendations for the Use of Antiviral Medications in the Treatment and Prevention of Influenza for the 2009-2010 Season September 8, 2009 2:00 PM ET; http://www.cdc.gov/h1n1flu/recommendations.htm

Neuraminidase inhibitor resistance (Oseltamivir™, Zanamivir™)

- Osteltamivir resistance is due to H275Y (histidine to tyrosine) mutation in neuraminidase of H1.
- >99% seasonal H1 now oseltamivir resistant
- 2 dozen cases of oseltamivir R in pandemic H1N1 (1 in Alberta, 1 in Quebec).
- No Neuraminidase inhibitor resistance in H3.

- Zanamivir resistance not described

Pyrosequencing to rapidly detect Oseltamivir R

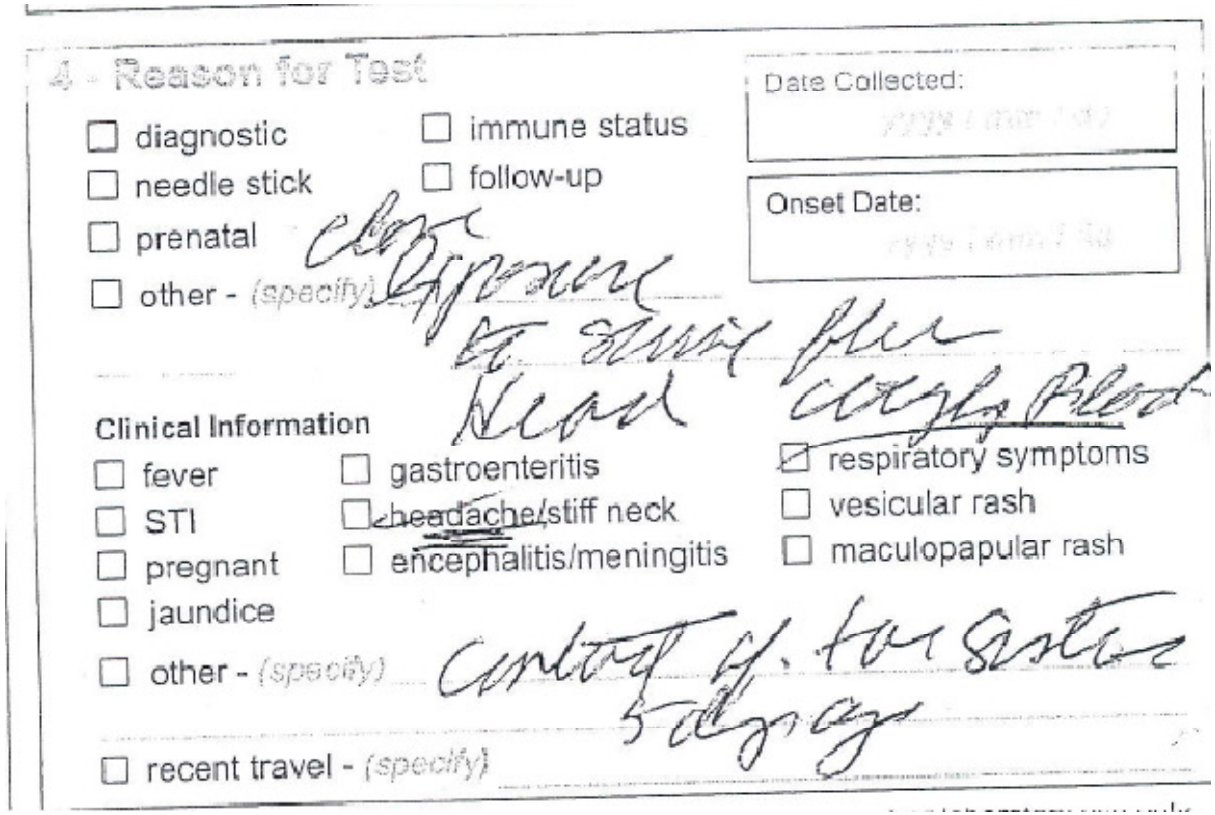


Whole Genome Sequencing

- Monitor for genetic drift and shift.
  - **Make sure current molecular diagnostic test is still working**
  - **Vaccine efficacy**
  - **Identify mutations that increase virulence.**

- Epidemiological tool
  - **Outbreak identification and tracking.**
  - **Early detection system for future pandemics**

You can't please all the people all the time…….



4 - Reason for Test

- ☐ diagnostic
- ☐ needle stick
- ☐ prenatal
- ☐ other - (specify)
- ☐ immune status
- ☐ follow-up

Date Collected:

Onset Date:

Clinical Information

- ☐ fever
- ☐ STI
- ☐ pregnant
- ☐ jaundice
- ☐ other - (specify)
- ☐ gastroenteritis
- ☐ ~~headache~~/stiff neck
- ☐ encephalitis/meningitis
- ☑ respiratory symptoms
- ☐ vesicular rash
- ☐ maculopapular rash
- ☐ recent travel - (specify)

Key Points

- Ambulatory patients should only be tested if at high risk of complications.
- Oseltamivir resistance is currently very rare in Pandemic Influenza A (H1N1) 2009.
- Therapeutic decisions should be made on clinical grounds without waiting for laboratory testing.
- Point of care tests should not be used in individual patients due to poor sensitivity.
- Bacterial coinfection should be considered and screened for in persons with severe pandemic (H1N1) 2009 infection.

Thanks To….

- **OAHPP: All Public Health Laboratory Staff**
  - Specimen Triage/DASH
  - Virus Detection
  - Molecular Diagnostics
  - Research Staff
  - Dr Goel, Dr Low, medical microbiologists, management team.

- **OAHPP:** Surveillance and Epidemiology , Emergency Operations.

- **Ministry:**
  - Dr King, Dr Williams
  - Emergency Operations
  - Public Health Protection & Prevention Branch
  - Public Health Division/Public Health Units