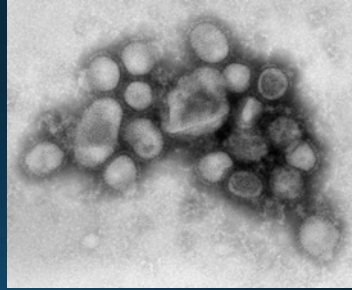


DPHL: Influenza Outbreak Surveillance



LPAC meeting
June 4, 2009




Influenza Background

Influenza A

- Classified by Hemagglutinin (H) and Neuraminidase (N) subtypes
- Current circulating seasonal strains are H1N1 and H3N2
- Human subtypes include H1N1, H3N2, H1N2, and H2N2
- Avian subtypes include H1 to H15 and N1 to N9
- Bird → human H5N1, H9N2, H7N7, H7N2, H7N3
- Swine subtypes include H1N1, H1N2, H1N7 H3N2, H3N1, H9N2
- Swine → human H1N1, H3N2


Influenza B

- Produces less serious disease than does Influenza type A
- Not categorized as by H or N type as is Influenza A



Circulating strains

- Northern hemisphere strains
 - A/Brisbane/59/2007 (H1N1)
 - A/Brisbane/10/2007 (H3N2)
 - B/Florida/4/2006 comes from B/Yamagata/16/88 lineage
 - B/Malaysia/2506/2004 comes from B/Victoria/02/87 lineage
- 2008-2009 vaccine included
 - an A/Brisbane/59/2007 (H1N1)-like virus
 - an A/Brisbane/10/2007 (H3N2)-like virus
 - a B/Florida/4/2006-like virus



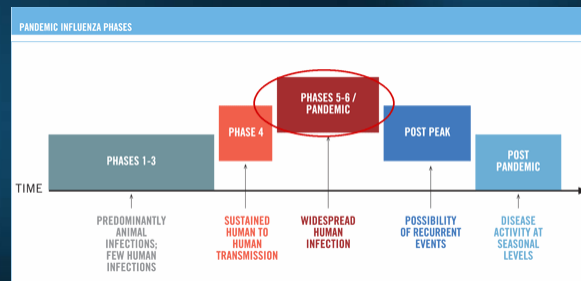
2009-2010 northern hemisphere vaccine recommendation

- A/Brisbane/59/2007 (H1N1)
- A/Brisbane/10/2007 (H3N2)
- B/Brisbane/60/2008-like

CDC working through the summer to develop and include a swine influenza component.


Why is Influenza A such a public health threat?

- antigenic drift (variation within the HN sub-type) or antigenic shift (variation between different HN sub-types) makes large portions of the population immunologically naïve on a regular basis
- Influenza epidemics have pandemic potential
 - Recipe for a human pandemic
 - » Emergence of a **novel sub-type** of influenza to which the population is immunologically naïve
 - » Replication in humans → disease
 - » Efficient human-to-human transmission




Reasons an influenza pandemic is unlike other public health emergencies or common disasters.

- Seasonal Influenza currently exists
- Inevitable
- Will arrive with very little warning
- Locally explosive epidemics
- Widespread, not focused like a bio-terrorism event
- Will put an extraordinary strain on human and material resources
- Effect will be relatively prolonged –weeks to months
average US winter epidemics affect 5% to 20% of the population with approximately 200,000 influenza-related hospitalizations during the 1990's and 36,000 influenza related deaths.




Preparations for 2008-2009 seasonal influenza

- Upgraded three ABI 7500 FAST instruments from “research use only” to “diagnostic” platforms
 - Stringent calibration, maintenance and performance specifications
- Validated the new FDA approved CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel) on the upgraded instruments
 - Primers and probes uniformly manufactured
 - Instrument functionality and assay reproducibility/precision were studied
- Acquired reagents
- Trained staff
 - 8 staff were trained



Preparations for 2008-2009 seasonal influenza


- Implemented algorithm
 - Perform real time RT-PCR for influenza A and B
 - Subtype influenza A using real time RT-PCR and batch influenza B for culture subtyping at the end of the season
 - Once influenza is established, automatically subtype any specimen received with a rapid positive A result
 - Complete electronic WHO report weekly
 - Submit representative specimens to the CDC



Implementing algorithm for 2008-2009 seasonal influenza

PCR vs Culture Considerations

- PCR: While the cost of primers is manageable, probes and reagents are very expensive. Culture: cell line maintenance time consuming
- PCR: The CDC primer sets are for H1, H3, and H5, not H1N1, H3N2, and H5N1 (no neuraminidases). The CDC is not overly concerned about this because it is the "H" (Hemagglutinin) that is related to pathogenicity. Culture: Turn around time is much longer
- PCR: Human RNase P cellular gene is used to monitor extraction efficiency and overall assay performance. Culture: Viral growth dependent on live virus



Lessons learned from Influenza A: H1N1/ Swine-Like: Outbreak Testing from the DPHL perspective

- Understanding the life of a specimen within the laboratory
- Mechanisms necessary for successful testing transitions
- Problems encountered

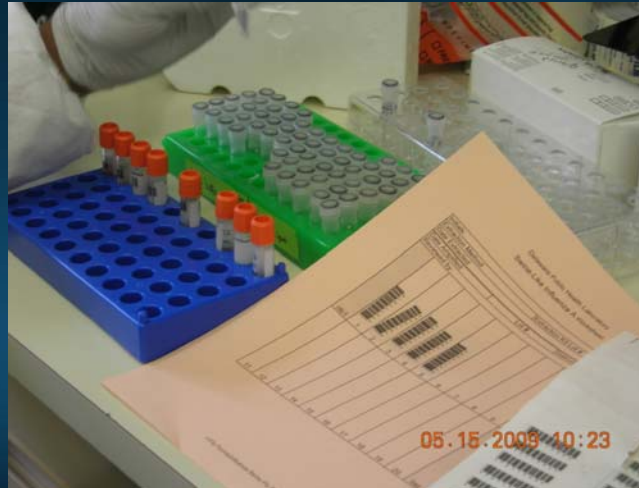
Specimen receiving



Specimen processing



Specimen extraction



Reaction preparation



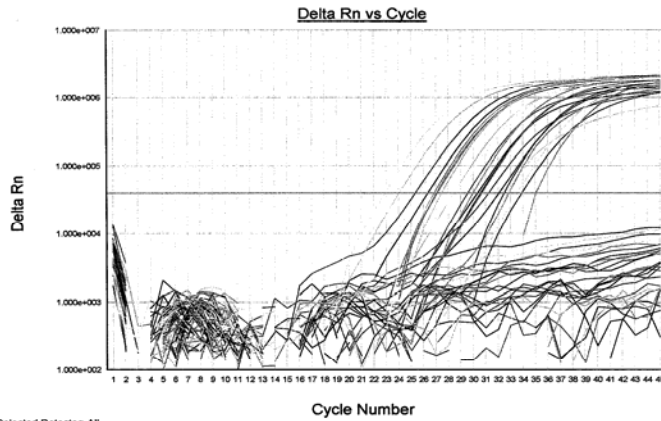
Reaction preparation



Nucleic acid amplification



Result analysis



Selected Detector: All
Well(s): A1-H12
Document: 050809SWFLU1CP (Standard Curve)

Result analysis

Well	Sample	Method	Result	Delta Rn	Cycle	Intensity
A1	InfA	FAM	(noise)	4.0e+004	3	15
A2	SW InfA	FAM	(noise)	4.0e+004	3	15
A3	sw HI	FAM	(noise)	4.0e+004	3	15
A4	RP	FAM	(noise)	4.0e+004	3	15

Well	Sample	Method	Result	Delta Rn	Cycle	Intensity
A2	F211322	InfA	Unknown	Undet.		
B2	F211322	SW InfA	Unknown	Undet.		
C2	F211322	sw HI	Unknown	Undet.		
D2	F211322	RP	Unknown	28.3871		
A3	F211323	InfA	Unknown	Undet.		
B3	F211323	SW InfA	Unknown	Undet.		
C3	F211323	sw HI	Unknown	Undet.		
D3	F211323	RP	Unknown	28.3927		
A4	F211324	InfA	Unknown	Undet.		
B4	F211324	SW InfA	Unknown	Undet.		
C4	F211324	sw HI	Unknown	Undet.		
D4	F211324	RP	Unknown	32.378		
A5	F211325	InfA	Unknown	Undet.		
B5	F211325	SW InfA	Unknown	Undet.		
C5	F211325	sw HI	Unknown	Undet.		
D5	F211325	RP	Unknown	27.0175		
A6	F211326	InfA	Unknown	Undet.		
B6	F211326	SW InfA	Unknown	Undet.		
C6	F211326	sw HI	Unknown	Undet.		
D6	F211326	RP	Unknown	22.8434		
A7	F211327	InfA	Unknown	Undet.		
B7	F211327	SW InfA	Unknown	Undet.		
C7	F211327	sw HI	Unknown	Undet.		
D7	F211327	RP	Unknown	26.2759		
A8	F211328	InfA	Unknown	Undet.		
B8	F211328	SW InfA	Unknown	Undet.		
C8	F211328	sw HI	Unknown	Undet.		

Reporting

Batch: PCR_FLU_SW-20090508-2 Resulted: *5/8/09*
 Reviewed: *5/8/09*
 Released: *5/8/09*

Batch Samples

Sample Number	Barcode ID	Date Received	Patient	PCR Status Result/Total	RESULT A/B	H1N3	RNP
1	291429	5/8/2009	PCR_NTC	R (P)	A B	H1 H3	RNP
2	291430	5/8/2009	PCR_N	R (P)	A B	H1 H3	RNP
3	291431	5/8/2009	PCR_P	R (P)	A B	H1 H3	RNP
4	291223	F211322		R (P)	A B	H1 H3	RNP
5	291225	F211323		R (P)	A B	H1 H3	RNP
6	291129	F211324		R (P)	A B	H1 H3	RNP
7	291128	F211325		R (P)	A B	H1 H3	RNP
8	291130	F211326		R (P)	A B	H1 H3	RNP
9	291131	F211327		R (P)	A B	H1 H3	RNP
10	291133	F211328		R (P)	A B	H1 H3	RNP
11	291162	F211331		R (P)	A B	H1 H3	RNP
12	291170	F211332		R (P)	A B	H1 H3	RNP
13	291308	F211333		R (P)	A B	H1 H3	RNP
14	291312	F211334		R (P)	A B	H1 H3	RNP
15	291315	F211335		R (P)	A B	H1 H3	RNP
16	291322	F211336		R (P)	A B	H1 H3	RNP
17	291324	F211337		R (P)	A B	H1 H3	RNP
18	291325	F211338		R (P)	A B	H1 H3	RNP
19	291267	F211339		R (P)	A B	H1 H3	RNP
20	291338	F211340		R (P)	A B	H1 H3	RNP
21	291301	F211341		R (P)	A B	H1 H3	RNP
22	291304	F211342		R (P)	A B	H1 H3	RNP

Sample Initials: *CMS* Extraction Initials: *GM* Amplification Initials: *CPD*
 Date: *5/8/09* Date: *5/8/09* Date: *5/8/09*

X_FluBatchSamples.rpt Page 1 of 1 Print Date: 5/8/2009

Transition from Seasonal to Swine Influenza

- Identification of unsubtypable Influenza A
 - Report issued as presumptive
 - Specimens shipped to CDC
- Confirmation by CDC
- Delegating duties
 - Data entry/packaging and shipping/testing
 - Reporting
 - Routine testing
- Creating shifts
 - Need to accommodate specimen receipt and result reporting
- Screening methods
 - Options to prevent becoming overwhelmed
- Obtaining swine influenza testing reagents and implementing new method
 - Emergency Use Authorization obtained from FDA
 - New method used new primer sets (human seasonal A, swine A, Swine H1, and RNP)
 - Results analysis
- Verification and Validation of testing
 - CDC confirmed specimens retested using new sine reagents and results/Ct values reported to CDC
 - CDC issued a validation/verification certificate to DPHL
- Developing new reports and tests in the computer system
- Implementing new reporting techniques
 - Getting information out fax/email/call
 - WHO reporting daily



Problems Encountered

- Changing/developing/evolving recommendations and guidelines
 - From acceptable specimens to safety requirements
- Sequestered reagents
 - Limited testing
- Public concern
 - Phone calls
- Specimen ID or CDC/DPHL/Epidemiology
 - Agencies assigned unique identifiers
- Getting specimens to the lab
 - Couriers added routes and stops
- Creating tests in the Laboratory Information Management System
 - Needed a new test created in order to generate reports and statistics
- Reporting
 - Obtaining results before specimen officially in LIMS system
 - Notification to submitter and agencies



Current statistics as of 5/29/09

Seasonal Influenza

- ~ 2300 tests completed
 - 373 Influenza A H1
 - 21 Influenza A H3
 - 209 Influenza B: Malaysia
 - 3 Influenza B: Florida

Swine Influenza

- 888 specimens tested
 - 133 positive Influenza A:H1N1/swine-like
 - 83 sites submitting specimens
 - Kent General 24.3%
 - Milford Memorial Hospital 14.1%
 - Center for Pediatric and Adolescent Medicine 6%
 - Christiana Care Medical Center 5.9%
 - A. I. DuPont Hospital for Children 5.4%
 - Westside Health 5.3%
 - Nanticoke Memorial Health 5.1%

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