



DELAWARE LABORATOR

SUMMER

2009



PREPARATIONS FOR THE 2009-2010 INFLUENZA SEASON

Jane Getchell, DrPH, Lab Director and Rebekah Parsons, Lab Manager I



Influenza Testing, 2008-2009

In preparation for the 2008-2009 influenza season, DPHL upgraded three ABI7500Fast real-time PCR instruments from “research use only” to “diagnostic (Dx)” platforms and validated a newly FDA approved CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel). To ensure sensitivity, specificity, and competency, all DPHL instruments, protocols, and testing performances were qualified by the CDC prior to use of assays on human specimens. DPHL received official verification and certification letters from the CDC. Using this assay, DPHL’s ’08-’09 seasonal algorithm consisted of rRT-PCR for Influenza A and B, the subtyping of Influenza A using rRT-PCR and the batching of positive Influenza B specimens for subtyping by viral culture at the end of the season.

On April 27th, the FDA provided an emergency use authorization to CDC and extended it to qualified laboratories to support the use of a rRT-PCR Swine Flu Panel for swine influenza detection. DPHL met the criteria for a qualified laboratory by having a microbiologist attend training at the CDC, and having been CDC certified to use the FDA approved diagnostic platforms. DPHL was therefore eligible to use the FDA-cleared swine flu assay. The following statement issued by FDA dictated that labs use specific reagents listed in the protocol

or risk the possibility of losing certification and the ability to perform the assay.

“THE FDA EMERGENCY USE AUTHORIZATION REQUIRES THAT ALL QUALIFIED DOMESTIC LABS FOLLOW THE INSTRUCTIONS FOR USE PROVIDED IN THE IVD PACKAGE INSERT, NO EXCEPTIONS. This includes performing testing using only the ABI 7500Fast RUO or 7500Fast DX platform.”

The new test consisted of four reactions per specimen including human influenza A, swine influenza A, swine H1, and RNaseP (control). All reactions had to be positive to issue a positive result. A negative result must contain only a positive RNaseP. All other combinations of results would elicit an equivocal result with the specimen being sent to CDC for further testing. Controls run with all reactions had to meet the acceptable criteria for a valid assay.

DPHL completed the validation of the swine influenza assay on May 4th and May 5th, submitted results to the CDC and received a confirmation letter certifying the lab’s qualification to perform confirmatory swine influenza testing. To accomplish the certification, staff had to learn a new testing algorithm and result analysis.

With CDC sequestering the reagents necessary to perform the real time PCR assay and the potential depletion of valuable resources,

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SPECIAL POINTS OF INTEREST

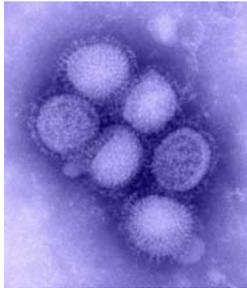
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2009-2010 Influenza Season, continued

Image of the H1N1 influenza virus (CDC).



the laboratory made the decision to cease routine seasonal influenza surveillance and focus solely on swine influenza testing. As of May 2, the lab tested 2353 specimens for seasonal influenza with 359 positive Influenza

A (94% H1 and 6% H3 subtypes) and 259 positive Influenza B (94% Malaysia subtype). After May 2, using the swine influenza testing protocol, DPHL was no longer testing for influenza B.

2009-2010 Influenza Surveillance begins in September

As of July 22nd DPHL tested 1478 specimens using the swine influenza assay with 417 swine influenza positive results. In early September the DPHL will switch from the swine influenza assay to a testing algorithm that will detect both influenza A and B as well as the various subtypes. DPHL will be able to distinguish the

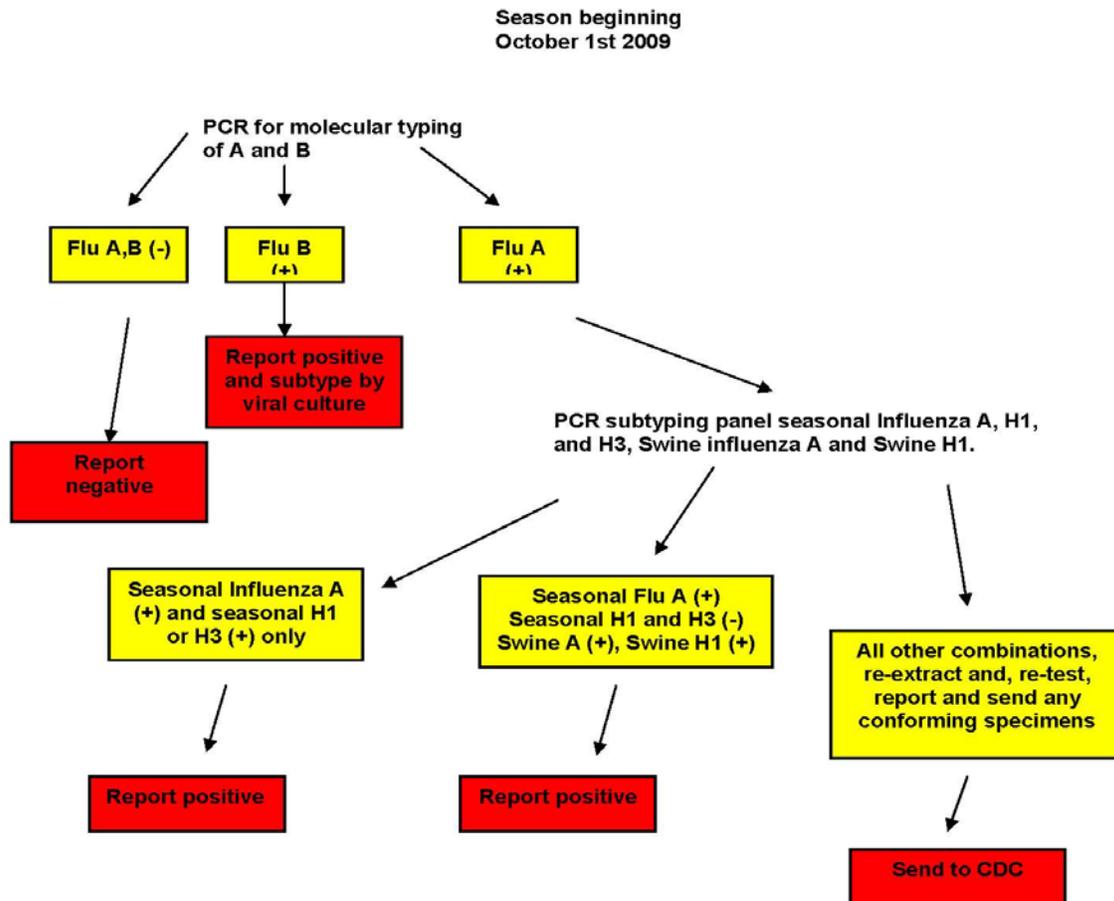
common human strains of influenza virus (H1, H3) from uncommon strains (H5 and H7) and from the novel H1 (swine-like) strain. The algorithm, shown below, may be adapted to suit detection demands and the predominant influenza strain in circulation.

The data generated by Real Time RT-PCR will help determine what influenza viral types are circulating in Delaware and will identify novel strains as soon as they appear in the state. Furthermore, test results can help determine the most appropriate treatment for patients. Reports including number of specimens typed as

Routine Seasonal Respiratory Virus Algorithm 2009-2010

Delaware Public Health Laboratory

Situation for use: Testing for the routine influenza through the sentinel physicians, hospitals, or outbreaks.



2009-2010 Influenza Season, continued

Influenza A H1, Influenza A H3, Influenza B, number of specimens not sub-typed and the age ranges of persons with positive results are submitted weekly to the World Health Organization. Specimens representative of the influenza season within the state are submitted to the CDC in an effort to assist in surveillance and vaccine formulations for the next influenza season.

Influenza testing is available to health care providers in Delaware at no charge. Results will be available within 48 to 72 hours depending on the number of specimens received.

We encourage health care providers who submit influenza specimens to DPHL to be on the laboratory's information management system (LIMS). This system will provide real time electronic access to test results. State clinics are already on LIMS. Interested health care providers need to sign a Memorandum of Under-

standing and receive 1-2 hours of training. Please call the DPHL LIMS Administrator at 302/223-1520 to initiate the process.

Laboratory couriers will pick up specimens daily from our regular pick-up locations. The laboratory will also make special arrangements for specimen pick-up for those practices that agree to be sentinel physician sites for influenza surveillance. An added benefit for sentinel sites is that the mechanism for receipt of specimen collection materials and pick up and delivery of specimens to the DPHL will already be in place should a new strain of influenza appear and only state public health laboratories have the ability to test for it as happened with the novel H1 (swine-like) strain earlier this year.

To receive a supply of collection kits please contact the laboratory kit room by calling 302/223-1520 or e-mailing **lab-**

supplies@state.de.us. Use the **influenza** virus detection kit for collection and transport of specimens for identification of influenza types A and B. Instructions are included in the kit and are printed below. If rapid testing has been performed, please provide the results on the requisition form. The requisition form can also be downloaded from our website: <http://www.dhss.delaware.gov/dhss/dph/lab/labs.html>.

For the latest CDC guidance on the use of rapid antigen detection tests for influenza see <http://www.cdc.gov/h1n1flu/>



INFLUENZA Virus Detection Kit - *Instructions*

Use this kit for collection and transport of nasopharyngeal specimens. Collect specimens as soon as possible after onset of symptoms.

Kit contains:

- 1 requisition form
- 1 screw-capped tube with 2ml of sterile transport media
- 1 flexible slender nasopharyngeal swab

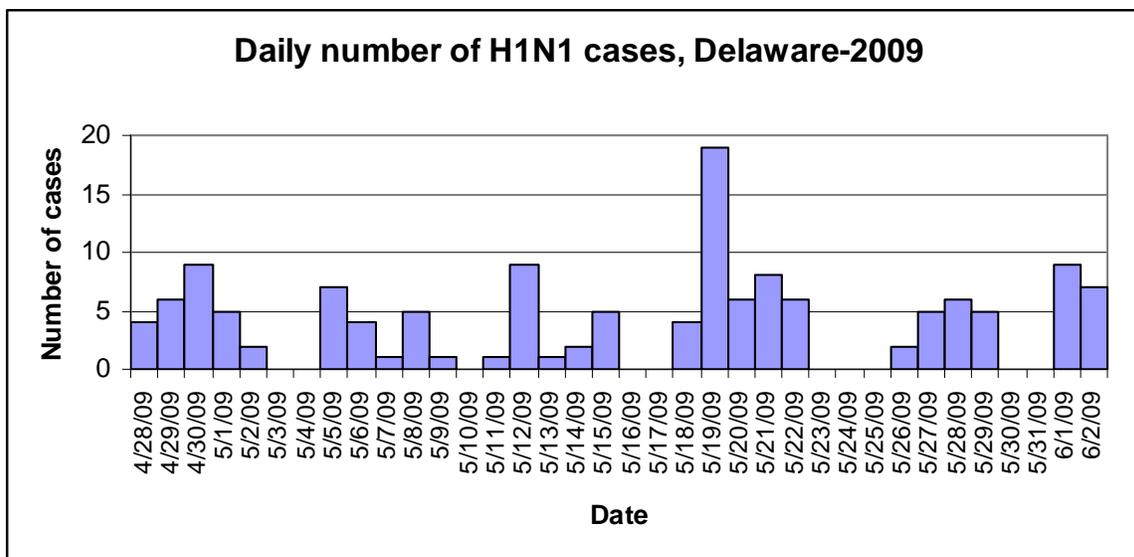
- Fill in *patient name* and *date collected* on tube.
- Complete requisition form. Failure to fill out all the requested information may result in delayed testing or specimen rejection.
- For collection of nasopharyngeal specimen insert the swab into the posterior nasopharynx and rotate it gently. Contact time should be 10 seconds or at least until the patient coughs.
- Place the swab into the screw-capped tube containing transport medium.
- Place collected specimens along with requisition slip back into original biohazard specimen bag.
- **Immediately place in a refrigerator (2-8 C). DO NOT FREEZE.**
- Arrange for transportation of specimen at 4 C (on ice or in cool van) to Virus Diagnostic Laboratory within 24 hours after collection. Courier specimen pick up locations can be found at the following web address: <http://www.dhss.delaware.gov/dhss/dph/lab/courieroutes.html>.

Finally, we ask that you be prepared to reduce the number of specimens you send at the peak of the season, traditionally in mid to late February. If we exceed our capacity for testing we will send out an e-mail giving instructions on numbers and types of specimens to submit.

We look forward to your participation in the Division of Public Health's Surveillance Program for Influenza.

WHAT IS NOVEL H1 (SWINE-LIKE) INFLUENZA AND HOW HAVE PUBLIC HEALTH AGENCIES RESPONDED TO IT?

Rebekah Parsons, Manager, Molecular Virology



Influenza A is classified by Hemagglutinin (H) and Neuraminidase (N) subtypes. The current circulating human seasonal strains are H1N1 and H3N2. Human subtypes of Influenza are known to include H1N1, H3N2, H1N2, and H2N2. Avian subtypes include all combinations of H1 to H15 and N1 to N9. Swine Influenza subtypes include H1N1, H1N2, H1N7, H3N2, H3N1, and H9N2. Influenza B is not characterized by H or N type and produces less serious disease than Influenza type A. The large number of potential recombinations of the Influenza A virus makes it a significant public health threat. Antigenic drift which is a variation **within** a single HN subtype, such as caused by point mutations within a viral sequence, and antigenic shift which is a variation **between** different HN subtypes, such as recombinations and reassortants between separate viruses, leave the population immunologically naive on a regular basis — hence the need for yearly vaccinations. Conditions for a human pandemic are ideal when there is an emergence of a novel subtype of influenza, the population is immunologically naïve, viral replication readily occurs in humans, and

transmission from human to human is efficient and sustainable. Novel influenza pandemics are unique from other public health emergencies or common disasters in that a seasonal influenza currently exists, its occurrence is inevitable and it will arrive with little or no warning. Locally explosive epidemics are expected as is widespread impact not focused in a single area. Effects will be prolonged from weeks to months causing tremendous strains on human and material resources.

Although there have been more than 50 cases of classical swine influenza in humans reported over the last 35 years, the triple reassortant swine influenza A (H1) virus combining segments of swine, avian, and human genes was seen in pig herds in the 1990's but not documented in humans until 2005. Discovery of a novel influenza virus occurs upon failed attempts to subtype with common influenza and genetic characterization by sequencing pinpoints and genetic drift or shift in the amino acids.

On April 17, 2009 the CDC and the California Department of Health detected **two** human cases of swine influenza A (H1N1) virus in San Diego

county California. Genetic characterization of these cases revealed that it was a novel virus not previously seen in swine or humans in the United States. Sequencing indicated that the emerging virus was a reassortant of North American and Eurasian swine viruses, and was a triple reassortant with avian, swine and human genes. Reports coming from Mexico indicated mass infection and possible fatalities. As public concern mounted, the CDC began a comprehensive analysis of these viruses and began updating testing protocols.

At about the same time seasonal Influenza surveillance was winding down, an alert went out to public health laboratories to be attentive to any positive results for influenza A, particularly strong positives, and negative results for the H1/H3/H5 subtypes. Since the seasonal influenza assay was designed for the detection of human H1 and H3 viruses, novel avian or swine strains were “unsubtypeable” or negative for any human subtypes.

Public Health laboratories were advised to contact the CDC regarding any specimen(s) indicating influenza A positive

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Novel H1 (Swine-like) Influenza, 2009, continued

and H1/H3/H5 negative (unsubtypable) and forward those specimens to the CDC immediately. Any commercial laboratories using the In Vitro Diagnostic (IVD) tests and detecting unsubtypeables were to contact their state public health laboratory.

The declaration of a global pandemic was issued on June 11, 2009 by the World Health Organization in response to the widespread reporting of cases (cdc.gov/h1n1flu/update). However as information about the reduced severity of the illness spread, public concern diminished as did the demand for testing. Nevertheless, in more than 15 weeks of testing for swine influenza, DPHL is still experiencing a steady test volume.

This outbreak emphasized the need for close interaction and collaboration between human and animal health

agencies. Also the numbers reported are only a small representation of the actual outbreak. Much of the population suffering from influenza like symptoms never seeks testing or treatment.

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[CDC - Influenza \(Flu\), Swine Influenza \(Flu\) Investigation](http://cdc.gov/swineflu/investigation.htm)". *Cdc.gov*. <http://cdc.gov/swineflu/investigation.htm>.

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NOTE: Rebekah Parsons' last day at DPHL was July 23. She left for employment in private industry and we are all sorry to see her go. We wish her the best in her new position.



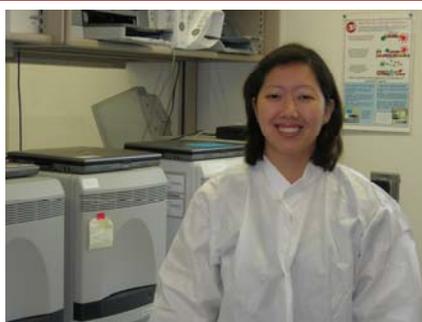
KENYAN DELEGATION VISIT

During July 14-16, 2009, in cooperation with the Association of Public Health Laboratories (APHL), DPHL hosted a small delegation of laboratorians from Kenya. While in Delaware, they attended a (brief) staff meeting to discuss our strategic plan, our goals and our accomplishments. The delegation was particularly interested in a review of the organization of laboratory services in the US, how they relate to public health and the role of the public health laboratory in the system. We also spent time discussing our QA/QC system and our LIMS system with them. They recently purchased the same LIMS system that we have and are very interested in seeing how we use it and how it works. We also hosted a BBQ luncheon in their honor which was attended by Deputy Secretary Henry Smith as well as public health representatives from other DPH sections. Dr. Smith gave a brief address to the group and handed out gifts contributed by the various DPH programs. The Public Health Preparedness section also presented the delegation with influenza prevention kits. Many thanks to all the programs who donated gifts; the delegation was thrilled to receive them.



Left to right: Wanga Michael Alba, Mamo Umuro Abudo, John Thiani Mwihia, Dr. Henry Smith (Deputy Secretary, DPH), Dan William Onyango Owiti, Ava Onalaja (APHL), Dr. Peter Mark Tukei, Dr. Jane Getchell (Director, DPHL), Dr. Siminyu Jane Francesca Wasike, Dr. Lucy Luta (Administrator, DPH Lead Program), Paulina Gyan (DPH, Office of Health & Risk Communication).

EMPLOYEE NEWS



Welcome to Amanda Chan, who joined the molecular virology team in June. She earned a Bachelor's from Virginia Tech in environmental science and a Master's at the University of California, Riverside, also in environmental science. Amanda's Master's thesis focused on environmental microbiology using samples from Don Juan Pond, Antarctica. She enjoys traveling, reading and crocheting. The molecular virology team is glad to have her on board!

Welcome to Sarah Beabout who started working at DPHL on June 8, 2009. Sarah is working through a summer fellowship program that was started this year with the University of Delaware Medical Technology Program and the Division of Public Health. The fellowship program provides the opportunity to learn state of the art technologies not offered in normal clinical rotations. Sarah is a graduate from Dover High School and will be completing her senior year at UD this fall, majoring in Medical Technology. She is an officer for the UD Field Hockey Club and also works as a certified phlebotomist at Crozer Chester Medical Center. Sarah is on the dean's list and a member of Phi Delta Epsilon Medical Fraternity and the Tri Beta National Biological Honor Society. Sarah will be working on several method validations for Norovirus and Cryptosporidia in foods through our Food Emergency Response Network grant with the FDA. The DPH Lab is lucky to have such a bright and talented person to spend her summer with us.



Meet our summer SPL intern Whitney Fitzwater! She is a junior Chemistry major attending Roanoke University in Salem, VA. While she has only been here a few months, Whitney has worked on a variety of different projects, including Water Microbacteriology testing and various Chemical Terrorism methods. Her main focus is working with Chemist Jacqueline Barnes to bring up the automated solid phase extraction system for pesticide testing in drinking water on the GC/MS. Whitney keeps herself extremely busy and works at WaWa in Middletown in the evenings and is an avid athlete who isn't afraid of a challenge!



DELAWARE'S DIVISION OF PUBLIC HEALTH LABORATORY

Delaware Public Health Laboratory
30 Sunnyside Road



Smyrna, DE 19977
302.223.1520
Fax: 302.653.2877

Built: 1990

Business Hours: 8 a.m. – 4:30 p.m.

Purpose: The Division of Public Health Laboratory currently offers consultation and laboratory services to state agencies, Delaware Health and Social Services and Division of Public Health programs including:

- HIV surveillance and prevention
- Immunization
- Lead
- Epidemiology
- Newborn Screening
- STD prevention
- TB Elimination
- Drinking water
- Preparedness



Karyl Thomas Rattay, MD, MS, Director,
Delaware's Division of Public Health

Christina Pleasanton, MS
Deputy Director, Delaware Public Health Laboratory

*"To Protect and Enhance the Health
of the People of Delaware"*

Jane P. Getchell, DrPH Director,
Delaware Public Health Laboratory

If you have questions regarding these articles or would like to receive a hard copy of this newsletter, contact the Delaware Public Health Laboratory at 302.223.1520. To receive this newsletter by email, contact liz.moore@state.de.us.

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