



# DELAWARE LABORATOR



Fall

2010

## MOLECULAR DIAGNOSIS OF INFLUENZA: HIGHS AND LOWS

*EMILY OUTTEN, MOLECULAR VIROLOGY LAB MANAGER*



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Prior to 2009 and the influenza pandemic of that year, the Influenza Division at the Centers for Disease Control and Prevention (CDC) had been preparing for just such an occurrence. While influenza is gradually and continuously changing, it was anticipated that a more rapid reassortment, also known as antigenic shift, would occur. CDC had already developed and distributed to public health laboratories a real-time reverse transcriptase polymerase chain reaction (rRT-PCR) method for the detection of influenza A, and B, and for subtyping of influenza A seasonal H1 and H3, plus the Asian lineage H5. This detection panel was known as the “5 target panel.” What nobody at CDC could have anticipated in 2009 was that the first specimens of the 2009 A (H1N1) pandemic would arrive while prominent scientists from the Influenza Division were at the annual “Clinical Virology Symposium,” in Florida, far from their Atlanta laboratory.

Nevertheless, on April 16, 2009, CDC determined that the preliminary sequence analysis of the first case suggested a new/ novel “Swine Flu”, different from previous U.S. isolates. By May 3, 2009, DPHL received the rRT-PCR reagents designed by CDC to test for this novel strain. Microbiologists at DPHL staggered shifts, curbed other testing, and were pulled from other sections to meet testing demands.

During that time frame, the Food and Drug

Administration issued an “Emergency Use Authorization,” which allowed DPHL to use the CDC’s rRT-PCR Swine Flu panel diagnostic test even though it was unapproved. While many laboratories had rapid influenza diagnostic tests, DPHL was the only Delaware laboratory permitted to use the CDC method and the only lab capable of subtyping influenza A specimens as 2009 A/H1. Additionally, rapid tests vary greatly in terms of sensitivity which can range from 20-70%<sup>1</sup>. Therefore, the rRT-PCR was capable of detecting and characterizing infections that may have been missed.

Over time, it was realized that the initial Swine Flu panel was not without minor flaws. Cross reactivity with avian strains and mutations in the swH1 target regions may have led to reduced sensitivity. A new and improved method was created, complete with new targets and the Swine Flu panel was discontinued. The new assay was named the “2009 A(H1N1) pandemic (pdm) panel.” DPHL was required to destroy the former Swine Flu reagents and kits. While the new 2009 A/H1 panel is much more specific and sensitive, it may still produce some low level amplification in those individuals who have recently received the Live Attenuated Influenza Vaccine, or FluMist®.

The Emergency Use Authorization for the original Swine Flu panel expired in June of 2010. However, the CDC received 510k clearance from the FDA for the new influ-

## ***SEVERE COMBINED IMMUNE DEFICIENCY (SCID): THE FIRST MOLECULAR NEWBORN SCREENING TEST***

***PATRICIA SCOTT AND CLOVER CARLISLE  
DELAWARE NEWBORN SCREENING LABORATORY***



Newborn screening laboratories across the country are beginning to gear up for screening using a molecular platform. Typically, molecular assays have been used as a second tier test for confirmation of disorders like Cystic fibrosis, not as a primary screening test. However, SCID, Severe Combined Immune Deficiency, testing utilizes molecular assays as the primary screening test, and SCID is very likely to be the next disorder added to the Delaware newborn screening panel. The decision to add SCID follows the recommendation in May 2010 by Kathleen Sebelius, Secretary of the US Department of Health and Human Services, for states to adopt the national uniform screening panel as recommended by the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC). This panel now includes SCID as a core disorder.

SCID is a group of disorders caused by single gene defects resulting in a combined immune deficiency. Prevalence is about 1/100,000. These disorders have profound defects in T-lymphocyte differentiation and function. Some, not all, have defects in B-cell or Natural Killer differentiation as well. There are 13 different genetic forms of the disorder and affected individuals have severe defects in humoral and cellular immunity<sup>1</sup>. While SCID is the most significant of the disorders identified when looking for decreased T-cell receptor excision circles (TREC) levels, several other disorders are likely to be identified, including DiGeorge Syndrome, Jacobsen Syndrome, or other unspecified T-cell lymphopenia. The overall incidence of T-cell lymphopenias requiring bone marrow transplant is 1/38,000<sup>2</sup>.

Two states are already screening for SCID. Wisconsin has been screening since January 2008 and Massachusetts since February 2009. In addition, New

York and California are presently in a pilot testing phase. The technology used for identifying SCID-related disorders involves the identification of TRECs. Wisconsin is using a real-time polymerase chain reaction (RT-PCR) molecular method with automated extraction looking for TRECs and  $\beta$ -actin. They have tested over 162,000 babies, with the first true SCID case identified recently<sup>3</sup>. Massachusetts also uses a RT-PCR molecular assay looking for TRECs and RNaseP, and has screened 123,812 infants with one SCID case identified. A SCID method using immunoassay is in development in New York looking for lymphocyte markers CD3 and CD45.

Treatment for SCID involves bone marrow transplantation from an HL-A identical sibling or haploid parental donor to avoid the graft vs. host disease. For best results, the transplant should be done before 3.5 months of life. According to statistics provided by Dr. Rebecca H. Buckley and Duke University Medical Center, of babies transplanted before 3.5 months of life, 45/48 survive up to 28 years (94%). Overall survival rates are 76% or 126 of 166. Most causes of death after transplant are due to viral infections, such as Cytomegalovirus, Adenovirus, Epstein-Barr virus, Parainfluenza 3, Varicella, Herpes simplex, etc. Without treatment, life expectancy is less than two years.

The process to add a disorder to the newborn screening panel in Delaware involves a comprehensive review of the disorder, treatment, and testing options, followed by a recommendation from the Delaware Newborn Screening Advisory Committee to add the disorder to the Newborn Screening panel. At the last Advisory Committee meeting, SCID was discussed at length, with convincing testimony coming from physicians involved in the treatment of SCID's patients and Mr. and Mrs. Sawyer, parents

of two children with SCID, Alex and Austin.

The story of Alex and Austin, both born with the deadly disease, is a heart-breaking one. Alex was undiagnosed until 9 months old and Austin was diagnosed at birth. Both have had successful bone marrow transplants, but comparing pictures of their first birthdays shows what words can hardly explain – Alex was on life support and in multi-organ failure while Austin is enjoying his cake. What a difference screening can and will make in the lives of a SCID-affected infant!

In October 2010, the Delaware Newborn Screening Advisory Committee unanimously voted to add SCID to the Delaware newborn screening panel. Dr. Louis Bartoshesky and Dr. Sally Millbury-Steen, co-chairs of the Delaware Newborn Screening Advisory Committee, will send a joint letter to Dr. Karyl Rattay, Director of Public Health, asking her to direct that SCID be added to the Delaware newborn screening panel. In anticipation of this directive, DPHL staff are gearing up for the addition of yet another disorder to the panel of 38 disorders.

National efforts are also under way to increase awareness and facilitate the addition of SCID to NBS panels. Two people from each state were funded by the Association of Public Health Laboratories to attend the "Newborn Screening for Severe Combined Immunodeficiency (SCID) – Implementation, Challenges and Updates" meeting held on October 27-29. Clover Carlisle from the laboratory and Mary Manson from newborn screening follow-up program attended this meeting. The knowledge they gained from the trip will be integral in helping develop a plan for implementing SCID screening in Delaware.

Currently staff are evaluating available

methodologies, limitations to implementation (i.e., availability of TREC standards and control materials), estimated costs of those technologies, staffing requirements, space requirements for molecular screening of this magnitude, and funding sources. Our goal is to have the test operational by the end of 2011.

According to preliminary data from the Children's Hospital of Wisconsin, a single baby with a late SCID diagnosis costs an average of \$2.2 million. Medical care for one baby with an early SCID diagnosis costs \$250,000. Testing the 70,000 babies born annually in Wisconsin for SCID as part of the routine newborn screening panel costs approximately \$350,000 (\$4-5 per test). According to Dr. Brokopp, Wisconsin State Lab Director, "The savings from one positive diagnosis pays for testing of all babies for the entire year".<sup>1</sup>

<sup>1</sup>Lisa Koubrynsky, MD, Emory University. Presentation on *Severe Combined Immunodeficiency Clinical Phenotype and Disease Process*, Newborn Screening for SCID: Implementation, Challenges and Successes Meeting, 10/27/10, Atlanta GE.

<sup>2</sup>Mei Baker, MD, University of Wisconsin School of Medicine and Public Health. Presentation on *Newborn Screening for SCID in Wisconsin: Where we Were, and Where we Are*, Newborn Screening for SCID: Implementation, Challenges and Successes Meeting, 10/27/10, Atlanta GE.

<sup>3</sup>[http://www.emediaworld.com/press\\_release/release\\_detail.php?id=902085](http://www.emediaworld.com/press_release/release_detail.php?id=902085), *WI Public Health Lab Identifies First SCID Baby Using New TREC Assay*, Silver Spring, MD, June 16, 2010.

## Alex Sawyer's 1st Birthday



On Life Support  
Diagnosed @ 9 months



## Austin Sawyer's 1st Birthday



Diagnosed at birth

### *Molecular Diagnosis of Influenza, continued from page 1*

enza 2009 A(H1N1)pdm panel. The term 510k clearance refers to the FDA's risk-based classification systems intended for use in the diagnosis of disease. The CDC was able to gain Class II classification, or "moderate risk" with special controls. The special controls include limited distribution to public health labs, and specialized training to those qualified labs.

In early summer 2010, qualified scientists from public health labs received training at CDC, and in July 2010 the CDC released the new FDA approved 2009 A/H1 assay. The CDC developed methods for the detection and characterization of flu viruses (both the 5 target, and the 2009 A/H1 panel) are highly sensitive and specific. Currently there are no molecular methods for the characterization of flu B, which is subtyped using hemagglutination inhibition, or HAI, on virus grown in culture.

Influenza testing at DPHL is performed according to the package insert without alteration. The algorithm for the 2010-11 flu season involves first screening for influenza A and B, and then subtyping

accordingly. There are some subtle differences between the 5-target and 2009 A/H1 panels. The only acceptable specimen sources for the 5 target assay are nasal swabs or nasopharyngeal swabs. The 2009 A/H1 panel is validated for both upper and lower respiratory tract specimens, including nasal washes, and bronchoalveolar lavages. DPHL's current specimen collection kits provide only a nasopharyngeal swab for specimen collection and submission. DPHL may accept other respiratory specimen types for testing; however results will be reported for research purposes only and not for patient diagnosis. Moreover, all flu specimens are required to be refrigerated and lysed within 72 hours of collection. DPHL will test and report specimens greater than 72 hours old for research purposes only.

Additionally, in the summer of 2010, DPHL received a PyroMark™ Q24 instrument, also known as a Pyrosequencer. The Pyrosequencer will be used to partially sequence the flu A neuraminidase gene to detect anti-viral

resistance to Oseltamivir, or Tamiflu®. Short validation runs thus far have proven to work well. DPHL plans to institute this testing in the coming months for all Delaware patients hospitalized with influenza A.

DPHL microbiologists have completed flu PCR training and are ready for whatever the flu season may bring. Thus far, it appears that seasonal H3N2 is prevalent and 2 cases have been confirmed in Delaware already. Come what may, the only sure thing will be change. CDC anticipates merging the two flu panel assays, validating more extraction platforms, and validating additional specimen types. DPHL will work with Delaware hospitals, reference labs, health clinics, schools, and sentinel physicians to provide information on the latest changes in testing and specimen submission.

#### References:

Georgia. Centers for Disease Control and Prevention, Influenza Division. [Influenza Viruses: Surveillance, Prevention and Control](#). Atlanta, CDC: 2010.



Dr. Linda Popels graduated on October 7, 2010 from the Environmental Leadership

Academy, a partnership between the Department of Natural Resources and Environmental Control and the Division of Public Health's Office of Performance Management. The program was a ten week Dale Carnegie based leadership training and included topics such as developing organizational leadership and putting enthusiasm to work. The objectives of the program are to develop skills to lead with vision, improve communication and create a positive environment where employees can reach their full potential.

As part of the Leadership Academy, all students were required to develop an

## ***ENVIRONMENTAL LEADERSHIP ACADEMY & LABORATORY ACCREDITATION***

### ***LINDA POPELS, PH.D., LABORATORY MANAGER I***

Innovation Project to improve an aspect of their job or performance. My innovation project looks at laboratory accreditation for Delaware state agency laboratories. Accreditation ensures that the laboratory reports accurate results through an independent evaluation of laboratory competence, including inspections and data audits. The Delaware Public Health Laboratory (DPHL) has accreditation through many different programs but lacks universal accreditation across all laboratory programs.

Accreditation is varied at other state agency laboratories. Many have accreditation programs that are required for their individual programs and a few have universal accreditation or are beginning the process of obtaining universal accreditation. But there are a few pro-

grams that have accreditation based on proficiency testing only, not laboratory inspections and data audits.

The DPHL received an Association of Public Health Laboratories Innovation Grant that will help us obtain International Organization for Standardization (ISO) 17025 accreditation for the environmental microbiology and chemistry sections. We are in the process of hiring a contractor to assist us with implementing the necessary steps to obtain accreditation. We plan to work towards universal accreditation in Delaware's laboratory system for those labs that are not already ISO17025 accredited, beginning with DPHL. We will develop a quality management/assurance template for use by other laboratories that may wish to adopt a universal accreditation system.

## BIOLOGICAL LABORATORY PREPAREDNESS ADVISORY COMMITTEE

**DEBRA RUTLEDGE, MBA, MT (ASCP), BIOTERRORISM COORDINATOR**

**MARION FOWLER, MT (ASCP), BT MICROBIOLOGIST**

**LINDA POPELS PH.D, MICROBIOLOGY LAB MANAGER**

**EMILY OUTTEN, BS, MOLECULAR /VIROLOGY LAB MANAGER**

The Biological Laboratory Preparedness Advisory Committee (B-LPAC) met on September 29, 2010 at the Delaware Public Health Laboratory (DPHL). The meeting was well attended and many of DPHL's internal and external partners were present. Last year, it was decided to split the committee into two separate groups. As a result there is a separate meeting for environmental and/or chemical partners coordinated by Tara Lydick, Chemical Terrorism Coordinator. The most recent meeting for the E-LPAC group was held on October 29.

The partnership between Delaware's sentinel laboratories and DPHL was discussed in detail. A CDC/Laboratory Response Network (LRN) Preparedness Grant of 2001 required the state public health laboratory to increase its ability to confirm and/or rule-out bioterrorism (BT) agents both by culture and molecular tests. The capacity of the lab was also increased to respond to a potential outbreak situation. The grant also required the state to improve its ability to recognize a terrorist threat or other emergency situation. In order to comply with this requirement and to improve communications and coordination among labs, LPAC was formed. Meetings include all Delaware hospitals (sentinel laboratories), the Division of Public Health, Preparedness, Epidemiology, Division of Natural Resources and Environmental Control, private laboratories, the 31st Civil Support Team of the Delaware National Guard, the FBI and other homeland security officials.

The most notable achievement between DPHL and its sentinel laboratories has been the increase of the sentinel laboratories' ability to rule-out or refer BT organisms to DPHL. As early as 2002, DPHL presented the first "Agents of Bioterrorism" workshop for Delaware microbiologists. The College of American Pathologists (CAP) prepared their

first laboratory preparedness survey soon after. Within a few years, DPHL was able to include a "wet workshop" in the afternoon portion of the Agents of Bioterrorism: Annual Sentinel Lab Update. CAP, with the help of APHL, reorganized their proficiency testing to include appropriate bioterrorism organisms and the name was changed to the Laboratory Preparedness Exercise (LPX). The CAP LPX also included instructions for notification of the state public health laboratory when unable to rule-out a BT agent. Packaging and shipping exercises were included with the CAP exercise to test the sentinel laboratories' ability to send a sample via a public health courier or outside non-DPHL courier. Both the workshop and CAP exercise testing results provide tangible proof of the knowledge gained by both the sentinel labs and the public health lab concerning bioterrorism organisms as required by the CDC/LRN Preparedness Grant of 2001.

A dramatic increase in the number of isolates sent to DPHL for rule-out of a BT agent has occurred. In 2009, 16 isolates were received from Delaware sentinel laboratories. By the beginning of September of this year, 27 isolates had been received. Since 2005, DPHL has received requests from sentinel labs for rule-out or confirmation of 42 *Bacillus anthracis*, five *Brucella* spps., five *Burkholderia mallei/pseudomallei*, three *Francisella tularensis* and one *Yersinia pestis*. Although not related to a BT incident, several *Brucella abortus*, *Brucella melitensis* and *Francisella tularensis* have been confirmed. Early detection of the possible BT agent by the sentinel labs and rapid confirmation by DPHL helps to determine the diagnosis and provide fast treatment of the patient. In the past, many of these organisms were reported as "unidentifiable gram negative coccobacillus or rod". Organisms also were cultured on the open bench in the

past. By increasing the awareness of both the sentinel labs and DPHL to this group of organisms and how to safely work with them, patients are receiving better care and laboratorians are less likely to contract a laboratory acquired infection.

The LPAC meeting also included an update on Tuberculosis (TB) testing. The talk focused on the Real Time PCR for TB, Molecular Detection of Drug Resistance (MDDR) testing at CDC and Quantiferon testing. This year DPHL began testing TB specimens using Real Time PCR. This technique has dramatically shortened the diagnosis time for TB from several weeks to 1-2 days. Highly suspect cases of TB must be communicated to the laboratory so the specimens are run as soon as possible. Molecular techniques are also used for rapid testing of drug resistance at the CDC. In special cases, specimens may be submitted to the CDC for MDDR testing. Examples of special cases include high-risk of RIF resistance or multi-drug resistant-TB and high profile patients such as nurses or day care workers. For a complete list of submission criteria and more information about MDDR, please go to <http://www.cdc.gov/tb/topic/laboratory/mddr.htm>

In 2009, the DPHL TB lab began Quantiferon interferon- $\gamma$  release assay testing at the request of the TB Program. It is now well established in our lab, and the clinics are relying upon us for this information. This test has significantly added to our workload as we performed over 1200 tests in 2009 and will surpass that number in 2010. This is a high complexity test that requires daily processing of specimens and at least 2 runs per week depending on workload and contact investigations. Our laboratory began using the Quantiferon assay to test for latent TB in international students at the University of Delaware in March

*Biological LPAC, continued from page 5*

2010. There were significant false positives with the PPD skin test due to the many international students who received the bacille Calmette-Guérin (BCG) vaccination. This required a lot of unnecessary documentation and tracking of patients who were not infected. Because the Quantiferon testing is not affected by past BCG vaccination, we have reduced the workload of the TB Control Program and resources can be best focused on active TB cases.

The upcoming influenza season was also a major topic of LPAC discussions. Emily Outten, Lab Manager of Molecular Virology, and Mardea Caulcrick, Epidemiologist both spoke on sentinel surveillance and detection and characterization of influenza. Mardea gave a power point presentation on the sentinel monitoring system in place in Delaware. Emily discussed this season's algorithm of screening for flu A or B, and subtyping by PCR for flu A and HAI on viral culture for flu B. For a discussion of acceptable specimens please see the "Influenza" article in this issue of the LabOrator. Some sentinel laboratories expressed concern with the 72 hour receipt time from collection, given that courier services do not exist over weekends, holidays, or evening hours. DPHL

assured those concerned that there would be no rejection of specimens greater than 3 days old, however, they would be considered off label and for research only. The molecular virology lab will continue to keep our sentinel labs informed of any changes.

Other infectious disease topics were also discussed. Kathy Wroten, infection preventionist from Christiana Care and Chair of Delaware's Hospital Acquired Infection Advisory (HAIA) Committee, gave a presentation on the new legislation requiring acute care facilities to report rates of certain infections. Debra Rutledge, infectious disease lab manager II, gave a presentation on Delaware's two VRSA cases and on a current DPHL study of HIV nucleic acid amplification testing. Tara Lydick, chemical preparedness coordinator, concluded the meeting with a packaging and shipping update. More information on this meeting and previous meetings, including copies of any handouts, can be found on our lab webpage. <http://www.dhss.delaware.gov/dph/lab/lpac.html>.

## DELAWARE'S DIVISION OF 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

## EMPLOYEE NEWS



*Jessica McKnight*

The laboratory welcomes Jessica McKnight and Dayle Bianco, who joined the laboratory in July 2010 as molecular biologists in the virology section. Jessica received a Bachelor of Science in Biology from the University of Scranton in Scranton, PA in May 2010. She enjoys long distance running, reading, and cooking with her boyfriend.

**Dayle** received a Bachelor of Science degree in Biology from Salisbury University in 2007. Before coming to DPHL, she worked as a laboratory technician for Merial Select, Inc. in Berlin, Maryland performing salmonella testing, virus titration, and other quality control duties. Dayle and her husband make gift baskets, entertain, and do anything water related.



*Dayle Bianco*

We're glad to have you, Jessica and Dayle!

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

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

Christina Pleasanton, MS  
Deputy 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, Editor, at [liz.moore@state.de.us](mailto:liz.moore@state.de.us).

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

