Delaware Laboratory

Zika Virus Testing at the Delaware Public Health Laboratory

by Emily Hanlin

DELAWARE HEALTH AND SOCIAL SERVICES
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Was it a mosquito or a different vector? This is one question that has been asked many times in an effort to identify and differentiate The possible origin of various infectious diseases over the years. Currently, people are aware that the Zika virus exists following the extensive national and local news media coverage that it has received. Some, however, are unaware that it is a mosquito-borne flavivirus that was first identified in monkeys, in Uganda, in 1947, through a network that was monitoring yellow fever. In 1952, it was found in humans living in Uganda and in the United Republic of Tanzania. Since then, outbreaks of this virus have been recorded in Africa, the Americas, Asia, and the Pacific.

The first large outbreak occurred in the Island of Yap (Federated States of Micronesia) in 2007. In mid-2015, the virus once more began to appear in the headlines after Brazil reported an association between Zika virus infection and Guillain-Barré syndrome. In Oct. 2015, Brazil also reported an association between Zika virus infection and microcephaly (small head) among children born to infected mothers. While the symptoms of Zika in a healthy individual tends to be mild, the risk to pregnant women can be significant as Zika can cause serious birth defects. In 2015, personnel at the Delaware Public Health Laboratory (DPHL) noticed increasing reports of Zika virus and suspected that this virus might be significant in the U.S. For this reason, laboratory personnel began to track its evolution in the literature.

On Jan.15, 2016, the Centers for Disease Control and Prevention (CDC) released a health alert announcing that Zika virus had been detected in travelers returning to the United States from abroad. This spurred concern among DPHL staff. Preparations were initiated for profile testing in the near future.

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Over time DPHL began to receive increasing phone inquiries about Zika virus testing because commercial laboratories offered testing for this virus, at that time. Fortunately, DPHL had already initiated use of real-time Reverse Transcriptase (rRT-PCR) methods for Chikungunya virus and Dengue virus, a test process that is similar to that used for Zika virus. Even so, DPHL had not yet formally implemented the diagnostic method, which consisted of rRT-PCR and Enzyme Linked Immunosorbant (ELISA) methods for the detection of IgM antibodies these were considered by the CDC to be strictly laboratory developed tests (LDTs), and few public health laboratories had access to the methods.

DPHL immediately mobilized and adopted the Zika LDT rRT-PCR test from the CDC. Because this test was an LDT, it required that DPHL complete a full validation study for accuracy, precision, analytical sensitivity, and analytical specificity. DPHL expedited reagent purchases and quantified virus samples were obtained to "spike" urine and serum specimens. Validation panels and positive controls were also purchased from the CDC. Success was achieved after two full-time employees and a lab manager worked intensely over a period of two weeks to implement the method, collate data, and write the study results. This became a record turnaround time for testing method implementation, at DPHL. Once completed, Zika testing capability was implemented for the State of Delaware.

On Feb. 9, 2016, the Office of Infectious Disease Epidemiology (OIDE) was contacted by the CDC to inform them that a female patient specimen previously shipped to CDC was confirmed positive for Zika. This confirmed that the Zika virus had made its way into Delaware via a traveler. Luckily, the patient was not pregnant and only presented mild signs and symptoms.

Within two weeks after DPHL had validated the LDT for Zika, the Food and Drug Administration (FDA) made effective the CDC Emergency Use Authorizations for the "Zika MAC-ELISA" and "Trio-plex real time RT-PCR Assay." DPHL decided to implement the FDA-approved methods since the Trio-plex method can be used to detects Chikungunya, Dengue, and Zika from serum specimens. Additionally, incorporating the MAC-ELISA allowed the laboratory to detect viruses in specimens that exceed the Zika viremia time-window. The virus can be detected after the first seven days following symptom onset. Zika virus specific neutralizing antibodies tend to develop toward the end of the first week of illness. These can last up to 12 weeks.

The effort at DPHL to adopt and implement both of the approved methods required that staff demonstrate a high level of organization and proficiency to successfully preform blinded testing panels. This was achieved and the CDC notified the DPH Molecular Virology Laboratory (MVL). Approval was issued to start diagnostic testing using the Trio-plex and MAC-ELISA, on April 1, 2016 and May 31, 2016, respectively. In Delaware, the decision to submit specimens for testing to DPHL is made by the OIDE. As of Aug. 31, 2016, the MVL has tested 141 Zika specimens; 62 specimens were tested using trio-plex rRT-PCR and 79 specimens were tested using MAC-ELISA.

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The MVL detected two positives using the LDT rRT-PCR for Zika and two by using the Trio-plex rRT-PCR. There have been a total of seven travel-related positive cases detected in Delaware as of June 30, 2016. Over this time, reference laboratories (LabCorp, etc.) have incorporated Zika testing into their operations. As of July 5, 2016, in Delaware, there have been seven positives, two indeterminate, one hundred and seven negatives and five pending.

As expected, no single laboratory method is without some limitations. While DPHL accepts serum, CSF, amniotic fluid, and urine specimens for Zika virus testing, only serum and CSF specimens are acceptable for the diagnosis of Dengue and Chikungunya virus using the Trio-plex. DPHL depends on the CDC to provide materials used in both methods even though those are, at times, in short supply.

While the CDC continues to work towards making improvements to the test method, the Trio-plex RNA extraction and purification process continues to be performed by hand. This is a time consuming process. The MAC-ELISA method takes a minimum of three days to complete and at times, may show cross-reactivity with other viruses. These may include cross reactivity with viruses from prior vaccinations against a flavivirus (e.g., yellow fever), previous infections with a related flavivirus, or a recent infection with a flavivirus.

While CDC can perform the tradititional Plaque-Reduction Neutralization Testing (PRNT) to measure virus-specific neutralizing antibodies that confirm primary flavivirus infection and differentiates Zika from other similar illnesses, DPHL does not have this capability.

Forthcoming changes to Zika virus testing will include automatic nucleic acid extraction from patient specimens and Zika viral antigen particles for the MAC-ELISA, which are easier to produce in large quantities.

MVL staff transformed their lab door to show where mosquitoes may breed and invited "Aedes albopictus" to be their "special guest."

Health care providers interested in having Zika virus testing done at DPHL can contact the OIDE at 888-295-5156. If you or your partner is of child-bearing age and sexually active, it is important to consult your health care provider before traveling to any area where Zika transmission can happen. Zika infection is sexually transmissible and can result in severe birth defects.

LPAC Spring 2016 Meeting Update

by Margaret J. Zimmerman

The spring meeting of the Delaware Laboratory Preparedness Advisory Committee (LPAC) was held at the Delaware Public Health Laboratory (DPHL) on Wednesday, May 4, 2016. A wide range of topics were presented, these include a presentation about radiation and how it affects our lives as well as an update about how the State of Delaware is preparing for a potential Zika mosquito season.

The first topic, presented by Office of Radiation Director Frieda Fisher-Tyler, was a brief overview of radiation science, the benefits of radiation, and why radiation can be a "tool of terror." Also discussed was the role of the Division of Public Health (DPH) in radiological emergencies.

The second presentation highlighted the dual agency effort to combat the emerging arbovirus, Zika. Dr. Bill Meredith, Environmental Program Administrator with the Department of Natural Resources and Environmental Control (DNREC), Division of Fish and Wildlife, discussed his agency's efforts to combat the threat of Zika by controlling the vector, *Aedes aegypti, through the* application of insecticides targeted to the vector and by educating the public on preventing mosquito infestations in backyards. Paula Eggers, DPH epidemiologist, shared the clinical features of Zika infections and the complications. Emily Hanlin, DPHL Laboratory Manager discussed the laboratory process for testing Zika virus and some of the challenges an implementing tests of emerging threats online quickly and effectively. Both agencies stand ready to face the mosquito season in Delaware.

A review of the 2015-2016 influenza season was presented by Emily Hanlin. The predominant strain this year was influenza A/2009 H1N1. The season should an overall positivity rate of 22 percent, which was high and might indicate a possible pre-screening of specimens sent to DPHL. DPHL made a request, to hospitals for specimen submissions to be randomized and for all influenza-like illnesses (ILI) to be submitted to DPHL, regardless of season. Summer submissions of ILI are important for the detection of novel influenza A, or strains like H3N2v that could be associated with swine.

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DPHL Biosafety Official Marion Fowler discussed the use of biosafety cabinets (BSC). Fowler shared the procedures including how to properly enter and exit from the BSC as to not disrupt the air curtain and cause materials to escape from the BSC. Also discussed was the upcoming biological risk assessments in all Delaware hospital laboratories. These assessments will start at the end of July, 2016. Finally, Fowler shared the date of the Bioterrorism Wet Workshop that will take place on Wednesday, Sept. 14 and Thursday, Sept. 15, 2016, at DPHL.

The meeting closed with a roundtable discussion among participants. Gregory Hovan, Laboratory Manager, shared information regarding the newly acquired gene sequencing instrumentation. Staff from Emergency Medical Services and the Office of Preparedness discussed upcoming workshops and table top exercises. The next LPAC meeting will take place during the fall of 2016.

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Is there Lead in Our Drinking Water?

by Clover Carlisle, Environmental Laboratory Manager
Sandra Sewitsky, Analytical Chemist III

Given the recent events in Flint, MI, and the extensive national media coverage that followed regarding high levels of Lead in public drinking water systems that may have affected people's health, the question that arises is: can Zika happen in Delaware?

In Delaware, there is a robust drinking water program that consistently monitors for potential contaminants. The Delaware Public Health Laboratory (DPHL) plays a significant role in helping to prevent an incident as what occurred in Michigan. The DPHL, Drinking Water Laboratory, is dedicated analyzing public and private drinking water samples for possible chemical and bacterial contaminants.

In ensuring the safety of residents and visitors in Delaware, the laboratory professionals who work in the Drinking Water Laboratory interact closely with the program staff in the Health Systems Protection, Office of Drinking Water (ODW) as well or with the U.S. Environmental Protection Agency (EPA) to certify the safety and quality of drinking water per the established national standards.

One of the test methodologies used is Inductively Coupled Plasma - Mass Spectrometry (ICP/MS). This is a sophisticated instrument for the detection of low levels of trace metals. These include lead, zinc, copper, barium, beryllium, arsenic, mercury, chromium, nickel, selenium, cadmium, antimony, thallium, uranium, and manganese. The ICP-MS method is a "well-established method promulgated by the U.S. Environmental Protection Agency (EPA) for the analysis of ground waters, surface waters, drinking waters, and wastewaters. The method was first published in 1990 to support the National Primary Drinking Water Regulations (NPDWR), … as part of the Safe Drinking Water Act (SDWA) of 1986." (Ref: https://www.perkinelmer.com/lab-solutions/resources/docs/APP_NexION300D-US-EPA200-8-Drinking-Water.pdf)

There are two main components to an ICP-MS. The first is the ICP pump, which injects a small portion of water sample into a stream of argon gas. This generates a water aerosol (mist) that is then exposed to a molecular ion plasma at 7,000 Kelvin (12,140 degrees F, 6,726 degrees C).

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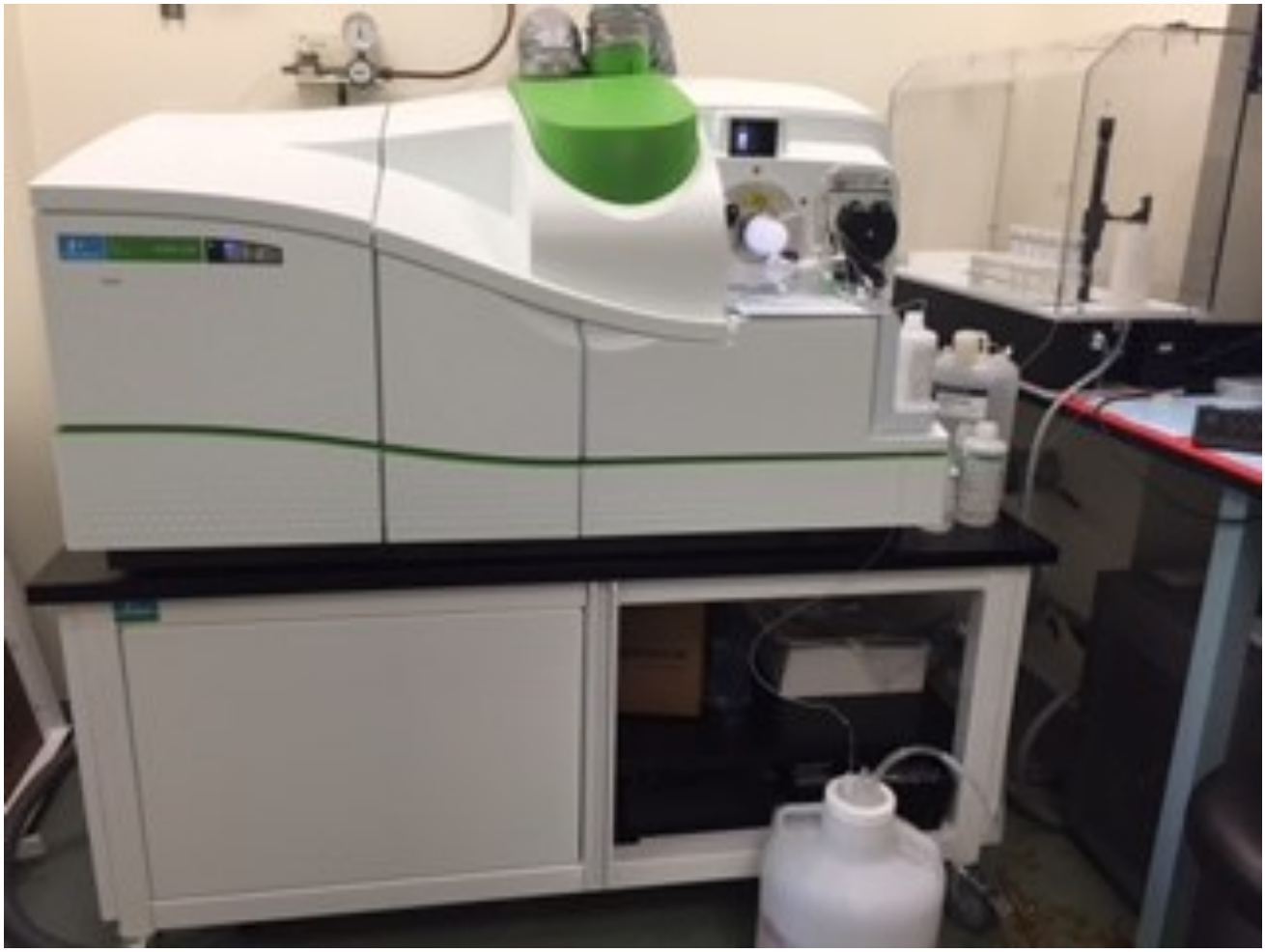
The temperature is comparable to the heat on the surface of the sun and creates positively-charged ions prior to moving the samples into the second component, which is the Mass Spectrometry (MS). The MS detects the concentration of ions by sensing their molecular weight and by electrical charge. It can detect metal molecules in small quantities (parts per billion), which is similar to a pinch of salt in ten tons of potato chips.

From these measurements, a calculation is done to determine the Maximum Contaminant Level (MCL), established by the EPA. The State of Delaware enforces this limit in drinking water. Should any water sample have a concentration of a metal higher than the MCL, the results are reported to ODW and procedures are initiated to ensure that measures are taken to lower the levels and protect the health and safety of residents and visitors to Delaware.



A Duck Odyssey

by Joshua Barth

The Delaware Public Health Lab (DPHL) welcomes *Anas platyrhynchos*, or Mrs. Mallard. Mrs. Mallard graced DPHL with her presence in late March. Unfortunately, she was only with us for the early spring season, departing in May. This is because she was utilizing our courtyard for a nesting site! Her nest was, directly under the window of the Molecular Lab (see photo below – look closely, she's well camouflaged). Mallard hens normally have a clutch size of eight to 13 eggs, which will hatch approximately 28 days after being laid. The 11 ducklings hatched May 3 and departed to the nearby lake on May 4. Mrs. Mallard and her brood appreciated the generosity of DPHL for allowing her family to utilize the courtyard. We wear many hats here at the lab (e.g. microbiology, virology, environmental chemistry, and molecular biology), now we can add another – wildlife management!

EMPLOYEE NEWS

5K Run/Walk
Governor's Cup

Several DPHL employees participated in the annual Governor's Cup 5K on May 18, 2016. Congratulations to Deidrhe Clayton who placed third in the female age group, 20 to 29.

DPHL participants in the 2016 Governor's 5K Run are (from left) Gregory Hovan, Emily Hanlin, Bobbi Turner, Deidrhe Clayton, and Anthony Eng.

EMPLOYEE NEWS

Charles Kosiek

DPHL is pleased to welcome Charles Kosiek, Jr. to the laboratory warehouse. Charles is a lab courier, covering Sussex County. Charles is an Army veteran and United States Postal Service retiree. He served two tours in Iraq in support of Operation Iraqi Freedom. While there, he earned his degree in applied electronic technology. Prior to joining DPHL, Charles was employed as a security officer at DHCI and the Holloway Campus. Charles is married with grown children and his interests include reading, his cat, and driving fast.

Sandra Sewitsky

DPHL welcomes Sandra Sewitsky to the drinking water laboratory. She earned her bachelor of arts with a major in chemistry from the University of Delaware. Sandy was previously employed as an analytical chemist in the Chemical Preparedness Laboratory at DPHL and worked at QPS, LLC for a year as an associate scientist involved in bio-analytical method development. In her spare time she enjoys cooking, fishing, and going to the beach.

www.dhss.delaware.gov/dhss/dph/lab/labs.html

Lori Bellotti

DPHL would like to welcome Lori Bellotti to the Molecular Virology Laboratory. Lori earned her medical technology degree from the University of Delaware and has worked as an off-shift generalist and specimen processing supervisor at Bayhealth Medical Center. She has also held positions at Old Navy, Blood Bank of Delmarva, the Delaware Division of Revenue, and currently owns and operates a home-based business. She is married and has a 9-year-old black lab named Raven. Lori's interests include boating, fishing, crabbing, riding motorcycles, watching movies, and sharing God's word.

Ed McGuire

DPHL Administration would like to congratulate Ed McGuire on his move to the Administrative Specialist I position. Ed previously held the position of supply, storage and distribution supervisor, overseeing courier and warehouse activities. In his new position, Ed will be performing data entry, greeting customers, and preparing conference materials, along with other administrative duties. His many years of laboratory experience is an asset in his new position.

Delaware Public Health Laboratory
30 Sunnyside Road
Smyrna, DE 19977
302-223-1520
Fax: 302-223-1520

Built: 1990
Business hours: 8:00 a.m. to 4:30 p.m.
Monday to Friday

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

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

Karyl Rattay, MD, MS, Director
Delaware Division of Public Health

Sergio Huerta, MD, 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 DPHL at 302-223-1520.

To receive this newsletter by email, contact Cathy Moore, office manager, at Cathyj.Moore@state.de.us.

"To protect and enhance the health of the people of Delaware"

EMPLOYEE NEWS

Randy Correia

DPHL welcomes Randy Correia. Randy is a Supply, Storage and Distribution Technician I and serves in the warehouse providing courier and warehouse coverage. He is married and lives in the Felton area and has three children. He retired from the Air Force Reserve and prior to coming to DPHL was the regional compliance manager for Walmart.

Joseph Heald

DPHL would like to welcome Joseph Heald. Joe is the newest Supply, Storage and Distribution Technician I who works on the northern courier route. Joe is married and lives in the New Castle area. He has a daughter and grandson and loves sports, travel, animals/wildlife, and spending time with his family.

EMPLOYEE NEWS

Dr. Keka Biswas

Dr. Keka Biswas joined DPHL in Dec. 2015 after teaching microbiology, chemistry, biochemistry and molecular virology at Delaware State University. She earned her Ph.D. in biology from the University of New Mexico. Later, she worked as Adjunct Associate Professor in the biology department at DSU. Currently, Keka is the bioterrorism microbiologist at DPHL. She works closely with the Sentinel Laboratory Program to identify special biologic microorganisms.

DPHL History Fun Facts

The Delaware Public Health Laboratory was created by the state legislature on March 23, 1899. At that time, the lab performed only pathological and bacteriological testing. As technology improved, so did the lab's capability to test for various bacterial and viral agents.

In May 1989 plans to build a new laboratory facility were underway. The new lab would be located on the grounds of the Delaware Hospital for the Chronically Ill in Smyrna. In 1990, the project was completed and the new lab was open for business. Since 1990, the lab has expanded its services by leaps and bounds. Today, the lab is able to perform DNA testing on various bacterial organisms, as well as amplified testing for Gonnehrea and Chlamydia.

In April 2005, the lab embarked on a new journey in testing. The lab officially opened the new Bio-level Safety II laboratory. These new capabilities enable the laboratory to be one of the few lead laboratories in the bacteriological identification and testing on the East Coast.

In March 2016, the lab moved into the 21st century of preventative medicine with the acquisition of the MiSeq, Next Generation DNA Sequencer. This instrument is used to identify the whole genome sequence of foodborne pathogens (i.e. Salmonella, Listeria, *E. coli*). This is a more sensitive technique for identifying. The technology allows for the identification of all genetic material (viral, bacterial, human) and will lead to new knowledge for public health testing.