Bureau of Public Health Laboratories - Continued Excellence in Service!

Salutations! We are pleased to present the 2018 Annual Report. 2018 has proven to be another great year for the Bureau of Public Health Laboratories (BPHL). BPHL’s continued success is based on strong partnerships, effective collaborations, and competent staff ensuring the fulfillment of our mission to “contribute to a healthier Florida by providing diagnostic screening, monitoring, reference, research and emergency public health laboratory services.”

This year has brought the following highlighted activities:

- Newborn Screening: The BPHL Newborn Screening (NBS) laboratory has added X-linked Adrenoleukodystrophy (X-ALD) to the panel of tests for all NBS programs.
- Next Generation Sequencing (NGS): The BPHL is transitioning from the traditional method of pulsed-field gel electrophoresis (PFGE) to whole genome sequencing (WGS) or NGS.
- Influenza-like Illness (ILI): The BPHL’s contribution to the 2018 extended ILI surveillance in Florida.

I am grateful for all the hard work of the BPHL staff who are committed to promoting and protecting the health of all Floridians. I am very proud to be a part of this dedicated team and look forward to celebrating another year serving the residents and visitors of Florida.

Patty Lewandowski, MBA, MLS (ASCP), CPM
Chief, Bureau of Public Health Laboratories

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The Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children and the Secretary of the U.S. Department of Health and Human Services work together to create the Recommended Universal Screening Panel (RUSP) which is a list of health conditions for which every baby in the country should be screened through testing. These disorders are reviewed for scientific understanding of the disorder and availability of treatment before recommendation to the Secretary of Health. The most recently added disorders are Pompe (2013), X-linked Adrenoleukodystrophy (X-ALD, 2015), Mucopolysaccharidosis Type 1 (MPS1, 2015) and Spinal Muscular Atrophy (SMA, 2018). Recent Florida legislation requires that disorders added to the RUSP must be evaluated by the Florida Newborn Screening (NBS) Advisory Committee within one year and once recommended for addition to Florida’s panel, the Newborn Screening laboratory must implement screening within 18 months. Planning for X-ALD screening was underway prior to passage of this legislation.

X-ALD was added to the Florida Newborn Screening panel in May 2018. In X-ALD, the protein responsible for transporting very long-chain fatty acids (VLCFA) into the peroxisomes (where they are degraded) is modified, resulting in accumulation of VLCFA in the white matter of brain, spine and/or adrenal cortex. X-ALD disorders fall into three categories: a childhood cerebral form, an adrenomyeloneuropathy type which affects spinal and peripheral nerves, and a form called Addison’s disease. The childhood form produces rapid neurologic decline due to severe inflammatory demyelination in the brain. These children undergo decline in development, with paralysis and death at an early age. An adult form of X-ALD presents with onset around 20 years of age; symptoms include difficulty walking, muscle spasms and peripheral neuropathy. Addison’s disease is the result of adrenal insufficiency. Decreased ability to produce cortisone by the adrenal cortex can precipitate a medical crisis resulting in death.

Since changes to the DNA for X-ALD only occur on the X chromosome, this disorder affects primarily males. Females have two X chromosomes and are protected if only one chromosome is altered, although some milder symptoms may occur in females as they age. In August 2017, work began in collaboration with PerkinElmer to provide a laboratory-developed test to allow for X-ALD screening until the PerkingElmer screening kit (NEOBASE2TM) was Food & Drug Administration (FDA) approved.

Laboratory renovation for new instrumentation and instrument...
Installation was completed in January 2018. Florida began reporting X-ALD for all babies in April 2018. The FDA approved the NEOBASE2™ screening kit in September 2018 and it will be ready for distribution to laboratories in December 2018. At that time, Florida NBS chemists will implement X-ALD testing using this kit.

Lysosomal storage disorders (LSD) are a group of genetic disorders resulting in buildup of metabolites in the lysosomes, causing a variety of symptoms and different ages of onset. Lysosomes are subcellular organelles that contain enzymes responsible for the breakdown of lipids and other large molecules in the cell. Two LSD approved by the RUSP, Pompe, and MPS1 were recently also approved by the Florida NBS Advisory Committee in August 2018.

Mucopolysaccharidosis Type 1 (MPS1) is divided into the severe and attenuated types. The enzyme affected is responsible for breaking down large sugar molecules called glycosaminoglycans. The inability to remove these sugars results in enlargement of the lysosomes and thus enlargement of the tissues. Generally, infants with this disorder do not show symptoms at birth, but the severe type manifests within the first year of life with developmental decline. Symptoms may include short stature and joint deformities, skeletal abnormalities, enlarged liver and spleen, and shortened lifespan.

Pompe disease has a more rapid and deadly onset in the infantile form. The enzyme deficiency in this disorder causes a buildup of glycogen in organs and tissues, especially in muscles. There are three types of this disease: classic infantile-onset, non-classic infantile-onset, and late-onset.

The classic onset is the most rapid onset after birth, with symptoms within months such as muscle weakness (myopathy), poor muscle tone (hypotonia), an enlarged liver (hepatomegaly), and heart defects. Without treatment, these infants may die in the first year of life.

The PerkinElmer NeoLSD™ MSMS Kit is a quantitative tandem mass spectrometry based assay that simultaneously determines the activities of six lysosomal enzymes. This kit must meet FDA approval before it can be used by NBS laboratories. The Jacksonville NBS laboratory has tested over 26,000 blood spots and provided data submission to FDA to evaluate the performance of the kit and associated instrumentation. The laboratory has completed the analysis with one MSMS instrument and is currently performing an instrument comparison to allow certification of a second platform to use the NeoLSD kit. This study should be completed in November 2018 and when approved, the laboratory will prepare to screen for MPS1 and Pompe.

Spinal muscular atrophy, is the most recent RUSP addition for Newborn screening. This genetic disorder affects nerves that control voluntary muscle; loss of motor neurons results in muscular weakness. A decrease in production of a protein essential for neuronal survival is the cause of this disorder. Weakness of muscles results in muscle atrophy and loss of function. There is variability in the onset of this disease with the most severe form impacting the SMN1 gene with disease symptoms manifesting themselves from birth to 6 months of age. Babies have a weak cry, breathing distress, muscle weakness, and fail to reach other developmental milestones. These babies have a poor prognosis for survival, but treatments are available now to prevent or delay symptoms. This disorder has not yet been added to the Florida Newborn Screening panel, however the NBS Advisory Committee will be reviewing this disorder in February 2019 for addition to the test panel.

“Operation of Automated Pipettor for processing 96 specimens simultaneously, used in many NBS methods."
BPHL and the Influenza Like Illness Surveillance Network

The Florida Department of Health (Department) maintains a robust influenza like illness (ILI) surveillance network, called ILINet. Year-round, but mostly during the influenza season of October to approximately May, the Bureau of Public Health Laboratories (BPHL) facilities in Tampa and Jacksonville receive specimens from patients matching ILI case definitions. These specimens come from primary care providers, urgent care centers, hospitals, clinics, and a variety of other sources. BPHL supplies these providers with all the sampling supplies and instructions they need to participate in this program; they also receive detailed laboratory tests reports for every specimen they submit.

These specimens are assayed for influenza virus, with influenza A or B positives being subtyped. The influenza A subtyping assay can differentiate routinely circulating viruses from suspect novel influenzas or new variants. These results are communicated electronically in real time to the Centers for Disease Control and Prevention (CDC) influenza group. A portion of the influenza positive specimens are forwarded to the CDC or a contracted reference laboratory for additional characterization. This testing includes whole genome sequencing (WGS) and antiviral resistance identification. Both the BPHL results and the extended testing performed by CDC are used to support interventions at a national level, and to contribute to annual influenza vaccine development.

In January 2018, the Department’s epidemiologists observed an increased level of influenza and ILI activity. The percent of emergency department and urgent care center visits for ILI at facilities peaked at the highest levels observed since 2004, the year this type of surveillance began. In response, the Department produced interim guidance on enhanced influenza surveillance for ILINet providers,
requesting additional specimens be collected and submitted above and beyond normal levels.

Additionally, the Department used resources already in place for ILI surveillance to reach out to hospitals and encourage them to submit already screened specimens from cases presenting in their intensive care units (ICUs). These cases were already screened as influenza A or B positive at the hospital and were sent to BPHL for additional typing and potential submission to the CDC. The ICU cases focused on patients under the age of 65, a demographic less commonly associated with severe manifestations of influenza. Performing supplementary testing, including WGS, on these cases helps support research into the pathology of these diseases. Since these specimens were already screened as influenza positive, this methodology also increased the laboratories chances of detecting a variant or novel influenza, something the originally testing hospital would have been unlikely to detect.

BPHL tested approximately 300 additional specimens related with the ICU cases. Additionally, BPHL tested at least 128 influenza pre-screened positive specimens per week for five weeks in a row in January and February 2018 to meet novel influenza event detection guidelines set by the CDC for Florida’s population size. This was the first year since implementing these guidelines in 2014 BPHL met this threshold. Fortunately, BPHL did not detect any variant or novel influenza viruses with this additional testing. This testing was funded through the CDC Epidemiology and Laboratory Capacity and CDC Public Health Emergency Preparedness grants. The Department learned the following from this:

- Using existing resources and systems in place for routine influenza surveillance can be leveraged to enhance surveillance.
- ICUs can be solicited in times of peak influenza activity to submit influenza positive specimens that help the state meet novel influenza event detection testing thresholds.
- BPHL needs to continue to recruit submission of pre-tested influenza positive specimens to be able to detect variant or novel influenza viruses.
JANUARY
Multiple Influenza-like Illness (ILI) Outbreaks

The BPHL Virology laboratories in Jacksonville and Tampa received samples from across the state for 18 Influenza-like illness outbreaks this month. The samples were mostly from assisted living facilities (ALF) and long-term care facilities (LTC). Detailed information is provided in the article “2018 Extended ILI Surveillance in Florida” included in this report.

FEBRUARY
Suspicious Substances – Biological Threat (BT) Laboratories

The BPHL-Tampa BT laboratory received three suspicious powder letters originating from Lake County. The Lake City specimens were three of 20-30 threat letters sent and all linked to a single perpetrator who was arrested in June 2018. An additional suspicious powder letter was received from Hillsborough County. Final results for all four samples were negative.

MARCH
Antimicrobial Resistance Laboratory Network

BPHL is a member of the Antimicrobial Resistance Laboratory Network (ARLN). This month, Florida sent 490 isolates to Tennessee’s State Lab (Southeast’s Regional ARLN laboratory) for testing.

APRIL
Gene Sequencing

The BPHL Molecular laboratory performed sequencing on HIV samples. HIV sequences are analyzed to determine resistance to antiretroviral drugs and for genotyping purposes. In addition, the Carbapenem Resistant Enterobacteriaceae (CRE) molecular assay, CARBA-R, was performed on 15 samples. Detailed information is provided in the article “Next Generation Sequencing” included in this report.

MAY
New Testing for Newborn Screening

The Newborn Screening (NBS) laboratory added X-linked Adrenoleukodystrophy (X-ALD) to the panel of tests for all NBS program specimens received by the BPHL. Detailed information is provided in the article “Latest and upcoming tests in Florida Newborn Screening Laboratory” included in this report.

JUNE
United States Naval Academy Interns

The BPHL-Miami laboratory welcomed three interns from the United States Naval Academy on May 31, 2018. Several BPHL (from all 3 laboratories) and Epidemiology staff gave presentations throughout the three-week internship. In addition, several field trips were taken, including a visit to the Federal Bureau Investigation headquarters, a visit to Homestead Airforce Base, and a visit from the Civil Support Team with their mobile laboratory.
JULY
Leveraging Technology via WebLIMS

The BPHL Mycobacteriology laboratory completed the web ordering project. This allows BPHL to receive lab orders through the web from external partners (outside of the department). Our first external provider was the Veterans Affairs Bay Pines Microbiology Laboratory in Tampa, Florida.

AUGUST
Rabies Testing Resumes in Miami

After a couple of years on hiatus, the BPHL-Miami laboratory started testing for rabies again. All three laboratories are now actively testing for rabies.

SEPTEMBER
Acute Flaccid Myelitis

The BPHL-Tampa laboratory received a CDC result of enterovirus A71 on a Cerebrospinal fluid sample from a child suffering from acute flaccid myelitis (AFM). This sample was originally tested at BPHL-Tampa as enterovirus positive and was sent to CDC for additional characterization. Enterovirus A71 has been associated with neurologic disease, including AFM, especially in children.

OCTOBER
Smallpox Discovery

The Biological Threat (BT) laboratory received a call about a box of old medical supplies with an item labelled “Smallpox Vaccine” that appeared to be several decades old. The BT staff opened the item and observed 15 sealed glass capillaries containing inoculating needles, and presumably the Vaccinia virus vaccine. The CDC Pox Virus laboratory requested the items to be shipped to CDC for further analysis. The CDC Pox Virus laboratory does not possess many strains of Vaccinia virus that predate the 1950s; therefore, virus isolation and whole genome sequencing will be attempted.

NOVEMBER
West Nile Virus

The Virology laboratories detected West Nile Virus (WNV) infections in many Florida counties this month. Experts consider WNV as endemic in the U.S. including Florida.

DECEMBER
Ukraine Visit

A delegation from Ukraine visited the Florida Department of Health from 12/10 to 12/14. The group came from several entities in Ukraine: CDC-Ukraine, Ukraine Public Health Center; the All-Ukrainian Network of People Living with HIV/AIDS; and the Kyiv City AIDS Center Laboratory. The group learned about HIV testing, HIV testing algorithms, HIV surveillance, linkage to care, HIV diagnosis and management in the community setting, and an introduction to laboratory information.
New Laboratory Technology: Next Generation Sequencing

Next Generation Sequencing (NGS) is a relatively new, promising technology that is revolutionizing public health laboratory science. NGS is sometimes termed Whole Genome Sequencing (WGS) because this technology can be used to sequence a microorganism's DNA at one time. Compared to first generation or "Sanger" sequencing technology that can only sequence smaller fragments of DNA in a single test, NGS/WGS can sequence multiple fragments at the same time and therefore produce more sequence reads in the less time. Table 1 highlights some of the major differences between first generation and next generation sequencing.

At the BPHL, laboratory staff perform multiple tests on patient samples to try to identify and characterize microorganisms that may be causing illness. One of the important areas of testing for the public health laboratory is the identification and characterization of bacteria that cause foodborne illness. Historically, the process for identification and characterization of these organisms was a complex set of tests including biochemical testing, serotyping, and a molecular fingerprinting method called pulsed-field gel electrophoresis (PFGE). [See the BPHL Annual Report from 2014 for an article on this testing process for Shiga-toxin producing E. coli (STEC)]. However, as technology has progressed, particularly in

Table 1: Comparing DNA Sanger Sequencing with Next Generation Sequencing

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<thead>
<tr>
<th>First generation &quot;Sanger&quot; sequencing</th>
<th>Next generation sequencing</th>
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<tr>
<td>Linear read of single DNA fragment</td>
<td>Parallel reads of millions of fragments in a genome simultaneously</td>
</tr>
<tr>
<td>Sequence gene (up to ~1,000 base pairs)</td>
<td>Sequence a whole microbial genome (megabases of reads)</td>
</tr>
<tr>
<td>Cost effective for small number of targets (e.g. single gene targets)</td>
<td>Cost effective for targeting a larger number of samples or a large volume of sequence</td>
</tr>
<tr>
<td>Identification of organisms</td>
<td>Identification and additional characterization of organisms (e.g. resistance and virulence factors)</td>
</tr>
<tr>
<td>Detection of mutations in genes associated with drug resistance</td>
<td>Detection of mutations in multiple genes simultaneously with higher resolution (e.g. detection of heteroresistance (subpopulations of resistant organisms))</td>
</tr>
<tr>
<td>Costly process if trying to sequence large amounts of DNA</td>
<td>More cost-effective for sequencing large amounts of DNA</td>
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What is a nucleic acid sequence? The succession of nucleotides (lettered A, C, G, T or U) that indicate the order of those nucleotides in a DNA or RNA molecule. These DNA or RNA sequences are what make up the genetic information in a living organism. The complete genetic material in an organism is called the genome. There are more than 3 billion base pairs [pairs of nucleotides] in humans, compared to the 4 million base pairs in an Escherichia coli bacterial cell.

What is sequencing? The procedure by which the order of nucleotides in a section of DNA/RNA is determined. This can be by any number of methods in the laboratory.
terms of molecular testing, this process can be accomplished with a single test, NGS. The NGS procedure sequences the DNA in the bacteria and sequence data are analyzed using various software tools to answer many different questions. This single test allows the organism to be identified, and for resistance and virulence factors to be determined. (Note: identification of resistance assists clinicians in determining which drugs will be most effective against the organism, while virulence factors can help determine how the organism is making the patient sick and can indicate how serious the course of disease might be). Importantly, sequencing data also enable one patient's illness causing organism to be compared to another patient's organism to determine if they are related. If organisms are related, an epidemiological investigation can determine if there is a common source which can help identify additional cases (outbreaks) as well as prevent further cases if the food source is identified and destroyed. This work is done at a local and state level, but the information is also fed into a national database that enables the detection of multi-state outbreaks of foodborne illness.

NGS is being implemented at all 50 state public health laboratories and some local public health laboratories. This new technology brings challenges with new equipment, new technology to understand, and new testing procedures to learn. However, the advantages of NGS are clear: it will improve efficiency in the laboratory; it will provide more in-depth information about the microorganisms causing disease; it will demonstrate their relatedness to identify links to outbreaks and potential outbreak sources. For Florida, this is essential. There is a high burden of enteric disease in Florida, with more than 5,000 salmonella cases identified each year and we don’t fully understand how people are exposed. This new technology will help improve surveillance and epidemiological investigations in Florida, ultimately leading to prevention of disease.


1. Association of Public Health Laboratories Annual Meeting, June 1-5, Pasadena, CA
   a. “Public health laboratory internship with the United States Naval Academy: Experiences of the inaugural year,” poster presentation: Stephen White
   b. “Developing the Partnerships, Exploring the Data and Sharing the Results,” Leah Gillis, Presenter
   c. “Florida’s Response to Natural Disasters”, Andrew Cannons, Presenter
   f. “Zika Epidemic: Developing Partnerships, Exploring the Data and Sharing the Results,” Leah Gillis, Presenter
   g. “Adventures in ETOR: 10 Years of ETOR – the Florida Experience,” Susanne Crowe, Presenter

2. National Environmental Monitoring Conference August 6, 2018
   a. “Florida Department of Health and Providers: A Partnership,” Vanessa Soto Contreras, Presenter

3. Public Health Information Network Conference, August 21, 2018

4. International Conference on Emerging Infectious Diseases, August 27, 2018

5. LRN National Meeting, September 6, 2018
   a. “Ebola in Liberia and Zika in Miami: Lessons Learned,” Stephen White, Presenter
   b. “BGs Stayin' Alive Bacillus atrophaeus (B. globigii – “BG”),” Phil Lee, Presenter
1. Association of Public Health Laboratories (APHL)
   a. Knowledge Management – Susanne Crowe
   b. Infectious Disease Committee – Marie-Claire Rowlinson (Chair)
   c. TB Subcommittee – Marie-Claire Rowlinson (Chair)
   d. Vector-borne Diseases Subcommittee – Lea Heberlein-Larson
   e. Workforce Development Committee – Leah Gillis (Chair)
   f. Biosafety and Biosecurity Committee – Andrew Cannons & Edgar Kopp
   g. Network of Laboratory Leaders Alumni - Andrew Cannons, Leah Gillis, Marie-Claire Rowlinson, & Mary Ritchie
   h. HIV/HCV Subcommittee – Berry Bennett
   i. Global Health – Stephen White
   j. Informatics – Marshall Cone & Susanne Crowe
   k. Public Health Preparedness and Response Committee – Andrew Cannons (Chair)
   l. Newborn Screening and Genetics in Public Health – Bonnie Taffe
   m. Newborn Screening Health Information Technology (HIT) Workgroup – Bonnie Taffe
   n. Sentinel Laboratory Partnerships and Outreach Subcommittee (SLPOS) – Philip Lee
   o. Food Safety – Patty Lewandowski

2. The NELAC Institute (TNI)
   a. NELAP Accreditation Council – Carl Kircher
   b. Laboratory Accreditation Body Expert Committee – Carl Kircher
   c. Laboratory Accreditation Systems Executive Committee – Carl Kircher
   d. Consensus Standard Development Program Executive Committee – Carl Kircher
   e. Proficiency Testing Program Executive Committee – Carl Kircher
   f. Chemistry Fields of Proficiency Testing Subcommittee – Carl Kircher
   g. National Environmental Field Activities Program Executive Committee – Carl Kircher
   h. Microbiology Committee – Vanessa Soto Contreras

3. Florida Public Health Association
   a. Board of Directors – Berry Bennett

4. Florida Consortium of HIV/AIDS Researchers
   a. Executive Board – Berry Bennett

5. Florida Viral Hepatitis Planning Group – Susanne Crowe

6. Biowatch Advisory Committee
   a. Jacksonville Biowatch Advisory Committee – Susanne Crowe
   b. Miami Biowatch Advisory Committee – Leah Gillis
   c. Tampa Biowatch Advisory Committee – Andrew Cannons

7. American National Standards Institute (ANSI)
   a. International Conformity Assessment Committee – Carl Kircher

8. International Organization for Standardization
   a. Technical Interface Group – Carl Kircher

9. University of South Florida
   a. College of Public Health Advisory Board – Andrew Cannons

10. U.S. Food and Drug Administration
    a. National Advisory Committee on Microbiological Criteria for Food – Patty Lewandowski

11. U.S. Department of Health and Human Services
    a. Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC), Laboratory Standards and Procedures Workgroup – Bonnie Taffe

Degree
Lea Heberlein-Larson awarded Doctor of Public Health (DrPH) degree from University of South Florida
Thank You for Your Service

45 Years
Mary Cook

35 Years
Corazon Angeles
Wayne Trasente

30 Years
Aurora Grospe
Sandra McConnell
Stephen Prudencio
Rhonda Shepherd

25 Years
Karen Chaires
Leila Filson
Dorcas Harper
Carl Kircher
Redentor Salonga
Virginia Simmons

20 Years
Neomi Abella-Sanchez
Keith Garrett
Lea Heberlein-Larson

15 Years
Leah Gillis
Derrick Harper
Walter Mock
Elesi Quaye
Mary Ritchie

10 Years
Najib Asghari
Ronald Brown
Patty Lewandowski
Tatiana Rodriguez

5 Years
Ronnie Banag
Calin Chiribau
Elizabeth Hasten
Linda Raab, Tampa
Jeremy Racicot
Ruth Dela Torre

Retirements

Vibha Mittal - Jacksonville Lab, 33 Years
Ligaya Antipolo - Jacksonville Lab, 31 Years
Estrelita Callao – Jacksonville Lab, 29 Years
Karen Chaires - Jacksonville Lab, 25 Years
Lilian Dela Torre - Jacksonville Lab, 23 Years

Josephine Griffin - Jacksonville Lab, 23 Years
Bonnie Hardy – Jacksonville Lab, 22 Years
Marielle Adamson – Tampa Lab, 19 Years
Dorothy Shaw - Jacksonville Lab, 19 Years
Bessie Clark - Jacksonville Lab, 15 Years