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Next Generation DNA Testing in Public Health Laboratory

by Greg Hovan, Microbiology Manager, Delaware Public Health Laboratory

Delaware Public Health Laboratory (DPHL) is embarking on a new wave of technology that protects the health of residents and visitors. The technology performs deoxyribonucleic acid (DNA) sequencing, a process that determines the precise order of nucleotides within a DNA molecule.

DNA sequences define microorganisms, such as disease causing bacteria and viruses. The DNA sequences tell us the organism's precise identity and whether it originated from a person, animal, food, or other source; whether the organism is genetically linked to other disease clusters; and what treatment might be most effective.

This technology comes about as a result of years of experience in DNA testing and puts DPHL at a higher level in infectious disease testing. The process is efficient, precise, and accurate.

DNA chains are formed by a set of basic building blocks called nucleotide bases. These include adenine (A), thymine (T), guanine (G), and cytosine (C). When bonded together, they form complementary amino acid "base pairs" (bp): A-T and G-C.

Long DNA chains are formed when these nucleotide base pairs link with one another to form base pair chains. The order or sequence of these base pair chains becomes the code that defines life, along with our external appearance such as eye color, skin texture and color, male or female, and height. It also defines how we function physically and physiologically, our predisposition to diseases such as cancer or autoimmune diseases, and many other biological traits. The entire sequence is referred to as the "genotype." Our human genome, or genotype, is comprised of about three billion base pairs.

DPHL has used basic sequencing methods for more than 10 years. In 2009, the Centers for Disease Control and Prevention (CDC) challenged public health laboratories to genotype Norovirus, otherwise known as the stomach flu. Laboratory professionals did just that and proceeded to define and implement other DNA testing applications, such as testing influenza virus mutations that caused resistance to antiviral therapy (Tamiflu) and identifying rare disease-causing bacteria.

As advantageous as DNA testing can be, the basic method was laborious, time consuming, and had inherent limitations. Among those limitations was that, on average, sequences are limited to about 600 base pairs. Although this is generally acceptable for identifying influenza antiviral resistance and some unknown bacteria, it is still a significant limitation given that many sequences are longer. If the entire human genome were to be sequenced by this process, it would take more than six years. Even so, it still allows for more rapid and more precise identification of infectious organisms.

For example, in 2016, there was a case

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involving an adult, who at a local hospital presented with cellulitis of a lower extremity resulting from lacerations and cuts obtained while cutting cement. The agent causing the cellulitis had invaded his bloodstream causing bacteremia. The hospital laboratory identified a gram-negative organism in two of four blood cultures. Further biochemical testing suggested that the organism was *Campylobacter.* Although *Campylobacter* is more often found as a food contaminant that causes gastrointestinal problems, it can also enter the blood stream and cause bacteremia, particularly if a patient is immune-compromised. The hospital then reached out to DPHL to confirm the findings. Microbiologists at DPHL tested for *Campylobacter* but the results were inconclusive since only a majority, *but not* all, of the biochemical tests suggested *Campylobacter*. The identity of the organism remained in question.

To resolve this puzzle, DPHL performed DNA sequencing analysis (with support from the CDC). The results showed the presence of a new organism that had not yet been classified. In early 2016, the CDC published this finding in an article that recognized a new genus and species - *Haematospirillum jordaniae*.

Sequencing technology has since evolved. In 2016, DPHL purchased a new sequencing instrument referred to as a "next generation sequencer." The MiSeq, or Whole Genome Sequencer (WGS), can sequence up to 80 million base pairs within several hours.

Since its implementation, the WGS has begun to replace Pulse Field Gel Electrophoresis (PFGE) methods used for foodborne pathogen surveillance. Delaware has benefitted from this technology. In the past two years, the CDC used it to clarify suspicion that farm produce being sold on Delmarva was the cause of hospitalizations following the ingestion of *Salmonella serovar* Newport. WGS revealed that the organism did not originate from Delmarva.

As compared to Pulse Field Gel Electrophoresis, Whole Genome Sequencers are much more sensitive in identifying organisms and help to geographically link outbreaks caused by organisms. This greatly improves the effectiveness of surveillance programs, such as foodborne outbreak surveillance, hospital acquired infection monitoring, antibiotic drug resistance monitoring, and identifying clinically significant, infrequent pathogens.

More recently, another development allowed testing of genetic materials recovered directly from clinical or environmental samples. This is referred to as metagenomic sequencing, environmental genomics, or community genomics. The process that allows for the testing of organisms directly from patient samples, without prior need to harvest the organism. This significantly reduces time to diagnose and treat. Beyond this, sequencing is used for direct testing of non-communicable disease forms, such as cancer and autoimmune diseases, for forensic investigations, and for the direct analysis of organisms in environmental samples.

DPHL continues to implement new DNA testing applications and to adapt to newer technologies for the benefit of Delawareans.

For more information, visit:
https://en.wikipedia.org/wiki/DNA_sequencing
Metagenomics, a guide from sampling to data analysis, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3351745/pdf/2042-5783-2-3.pdf
Haematospirillum jordaniae gen. Nov., sp. Nov., isolated from human blood samples, http://link.springer.com/article/10.1007/s10482-016-0654-0

Sequencing Technology and Terminology. Jackson Buss, Ph.D. Harvard Medical School,

Bioterrorism Lab Preparedness and Training Update

by Dr. Keka Biswas, Microbiologist II

In September 2016, bioterrorism (BT) training workshops were held for all of Delaware's sentinel laboratories. For many of the sentinel labs, the course served as a refresher and review of bioterrorism agents. Review of the different BT agents, modes of transmission, and presentation of disease were discussed and their biochemical reactions and colonial morphology. Case studies were distributed and discussed. The Centers for Disease Control (CDC) "Bioterrorism Response Guide for Clinical Laboratories" was used to determine which BT agent was the causative organism and when it would be necessary to perform additional tests prior to referring the organism to the Delaware Public Health Laboratory (DPHL).

One of the main goals of the bioterrorism training workshop was to help reinforce the habit of considering the possibility of BT agents when microbiologists are reading their culture plates or working on positive blood cultures. This is extremely important in the event of a possible covert attack in the U.S., as sentinel laboratories would probably be the first to see the agent in their lab. The sooner the microbiologist realizes that a BT agent cannot be ruled out by their lab, the sooner the organism can be referred to DPHL. Real-time, Polymerase chain reaction (PCR) can be performed to rule out the agent in question. Although PCR results are still considered preliminary, with culture required for confirmation, a positive PCR result would prompt state health officials and epidemiologists to begin case surveillance. Laboratory Response Network (LRN) culture confirmation procedures for a BT agent are set up as soon as the unknown organism reaches DPHL. Prompt referral to DPHL saves time and possibly patient lives, and protects sentinel lab workers from exposure to infectious organisms. Many organisms require Biosafety Level 3 (BSL3) laboratories and working practices. Working with all suspect organisms sent for rule-out should be suspended at the sentinel lab until DPHL rules out the BT agent in question.

If a BT agent is confirmed, whether it is from a naturally occurring incident or a true BT event, the organism should be autoclaved and disposed of properly per Select Agent Regulations.

Since the September 2016 workshops, DPHL had multiple opportunities to test the system for rule-out or referral of possible bioterrorism agents. The testing is a good measure for preparedness.

The sentinel lab recognized the colonial morphology and growth pattern, gram stain smear results, and other relevant characteristics and followed proper protocol by immediately contacting DPHL. Accurate and timely identification of this organism served to assess our PCR capabilities and culture confirmation for several BT agents. DPHL provided timely service to the sentinel laboratory to allow for a correct diagnosis and proper treatment.

Additionally, the Laboratory Preparedness Advisory Committee meeting met on Oct. 6, 2016 in Smyrna, Delaware at DPHL. The meeting was well attended by partners from the Division of Public Health, Civil Support team, and sentinel microbiology laboratories. There was significant discussion and contribution by people in the group. Presentations included weapons of mass destruction by the FBI, Zika updates, flu season planning, discussion on epidemiology and laboratory capacity for infectious diseases grants, laboratory preparedness exercise, and sentinel laboratories.

On a related note, DPHL successfully participated in two Food Emergency Response Network (FERN) high-risk proficiency testings, one in September 2016 and the other in March 2017. Proficiency testing was conducted on infant formula for multi-select BT agent, testing for ricin, *Bacillus anthracis*, *Brucella* spp, Francisella tularensis, and Yersinia pestis. In March 2017, FERN Proficiency testing was conducted on carrot juice samples for Franciscella tularensis. An LRN proficiency test panel of environmental and clinical samples was tested for bio-threat agents in January 2017.

Antimicrobial Resistance Laboratory Network

by Debra Rutledge, MBA, MT (ASCP), Infectious Disease Laboratory Manager, DPHL

Antimicrobial Resistance (AR) is a major concern worldwide. The United States has begun working on strategies to combat AR. The CDC is actively involved in these strategies along with many other state and federal partners, including health care providers, veterinarians, farmers, and environmental specialists. In the U.S., over two million infections and 23,000 deaths are due to AR every year.

Currently, there are four core actions identified to combat AR:

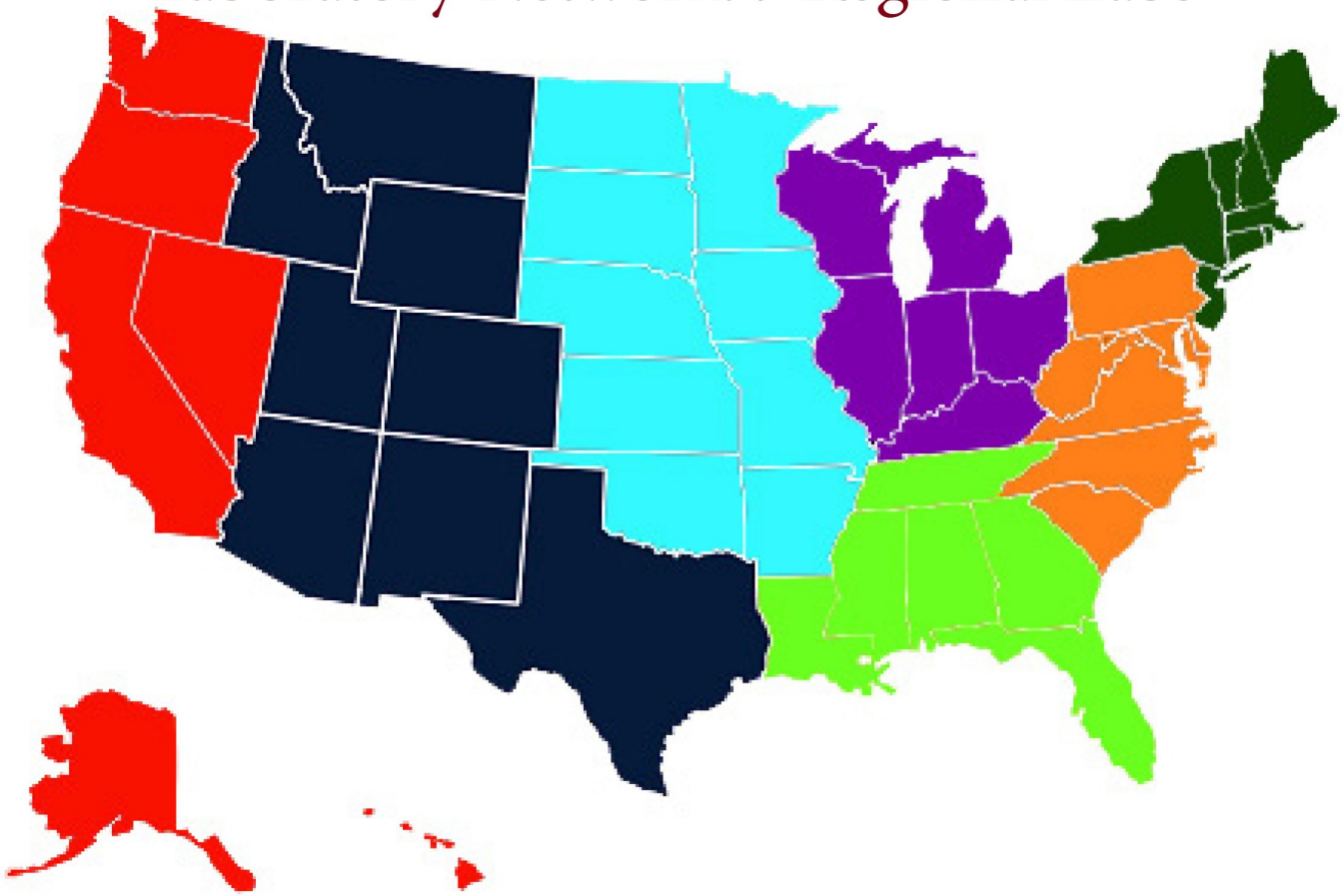
1) Preventing infections and preventing the spread of resistance
2) Tracking
3) Improving antibiotic prescribing/stewardship
4) Developing new drugs and diagnostic tests.

In December 2016, the CDC provided funding for the Antimicrobial Resistance Laboratory Network (ARLN) through the Epidemiology and Laboratory Capacity Cooperative Agreement. The ARLN is comprised of seven regional laboratories that provide testing and response for resistance organisms in human samples. These regional laboratories work with all state laboratories and five local laboratories in major cities to provide surveillance and confirmatory testing for AR organisms. The state and local laboratories also work with laboratories within their geographical areas to screen and confirm resistance mechanisms on target microorganisms. The regional laboratories perform additional testing to track outbreaks and work with infection control providers and epidemiologists to control the spread of resistant organisms. Each regional laboratory is working on specific resistance testing. (See map on page 5 for regional laboratories and additional AR capabilities.)

DPHL is currently validating several culture confirmation and molecular detection methods for carbapenem resistance (CR). These methods will improve response to outbreaks and speed up CR. DPHL will be working with laboratories to request isolates for screening and confirmation for these resistance mechanisms. Currently, the focus is on carbapenem resistant *enterobacteriaciae* and carbapenem resistant Pseudomonas aeruginosa that is resistant to at least one carbapenem. DPHL will confirm the identification of the isolate, perform antimicrobial susceptibility testing, and perform a phenotypic method to detect carbapenemase production using the carbapenem inactivation method. Several real-time Polymerase Chain Reaction methods will be run to test for resistance targets (blaKPC, bla NDM, blaVIM, and OCA-48 like genes). DPHL will also implement a bacterial isolate storage for AR if further testing is needed. The testing began in April 2017.

Contact Debra Rutledge at 302-223-1520 for more information or visit https://www.cdc.gov/drugresistance/index.html.

CDC Antibiotic Resistance Laboratory Network: 7 Regional Labs



WEST
Washington State Public Health Laboratories
- ✔ Core testing
- \+ Candida
- \+ N. gonorrhoeae

MIDWEST
Wisconsin State Laboratory of Hygiene
- ✔ Core testing
- \+ Reflux Culture Pilot
- \+ S. pneumoniae

MID-ATLANTIC
Maryland Public Health Laboratory
- ✔ Core testing
- \+ N. gonorrhoeae

MOUNTAIN
Texas DSHS Laboratory
- ✔ Core testing
- \+ N. gonorrhoeae

NORTHEAST
Wadsworth Center Bacteriology Laboratory
- ✔ Core testing
- \+ Candida

CENTRAL
Minnesota Department of Health PHL
- ✔ Core testing
- \+ Candida
- \+ C. difficile
- \+ Reflex Culture Pilot
- \+ S. pneumoniae

SOUTHEAST
Tennessee State Public Health Laboratory
- ✔ Core testing
- \+ Candida
- \+ N. gonorrhoeae
- \+ Reflex Culture Pilot

ARLABnetwork

National Quality Indicators in Newborn Screening

by Pat Scott, MTBT, Newborn Screening Laboratory Manager, Delaware Public Health Laboratory

The goal of the Newborn Screening Technical assistance and Evaluation Program (NewSTEPs) is to identify quality improvement initiatives and develop an innovative data repository and technical resources used by newborn screening programs. It is funded through a cooperative agreement with the Association of Public Health Laboratories (APHL) by the Genetic Services Branch of the Health Resources and Services Administration (HRSA).

Several quality improvement initiatives identified through NewSTEPs address timeliness in newborn screening (NBS). This effort followed a revealing Nov. 16, 2013 article published in the *Milwaukee Journal Sentinel*, "Deadly Delays," by Ellen Gabler. The article was based on data obtained through a massive FOIA request for information from all states. The findings led to the notion of setting national NBS standards and became a priority for the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC). The ACHDNC sent its recommendations to the U.S. Secretary of Health and Human Services. (See Figure 1.)

Figure 1. ACHDNC's Timeliness Recommendations

- Presumptive positive results for time-critical conditions should be communicated immediately to the child's health care provider but no later than the fifth day of life.
- All presumptive positive results for all other conditions should be communicated to the child's health care provider as soon as possible but no later than the seventh day of life.
- All NBS results should be reported within the first seven days of life.

To achieve these goals and reduce delays in newborn screening:

- Initial NBS specimens should be collected in the appropriate time frame for the baby's condition but no later than 48 hours after birth.
- NBS specimens should be received at the laboratory as soon as possible; ideally within 24 hours of collection.

Source: NBS Testing Program

To meet the designated timeliness goals, funding was provided through the NewSTEPs 360° grants of 2016 and 2017.

Delaware is a member of the New York Mid-Atlantic Consortium, which received a NewSTEPs 360° grant in 2016.

Data submission requirements under the NewSTEPs 360° grant requirements include monthly state-specific data for each quality indicator, QI1 to QI8. The data is uniquely structured so that everyone submits state-specific data in the same format. This has allowed for the compilation of national statistic, and blinded state-to-state comparisons.

Part of the change process at DPHL involved rebuilding the internal Quality Assurance monitoring program to include the national indicators. The data system, MSDS III, by Neometrics, a Division of Natus, was updated to include custom queries. Delaware led the way in working with Natus to establish the NewSTEPs system queries and to verify

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Figure 2. Time Elapsed from Birth to Collection

SPECTYPE	1	
Row Labels	**COUNT**	**PERCENT**
(01) Less than 12 hours	7	0.73
(02) 12 to 24 hours	19	1.98
(03) 24 to 48 hours	834	86.78
(04) 48hrs to 72 hours	53	5.52
(05) More than 72 hours	21	2.19
(06) Unknown	27	2.81
Total	**961**	**100**

Source: NBS Testing Program

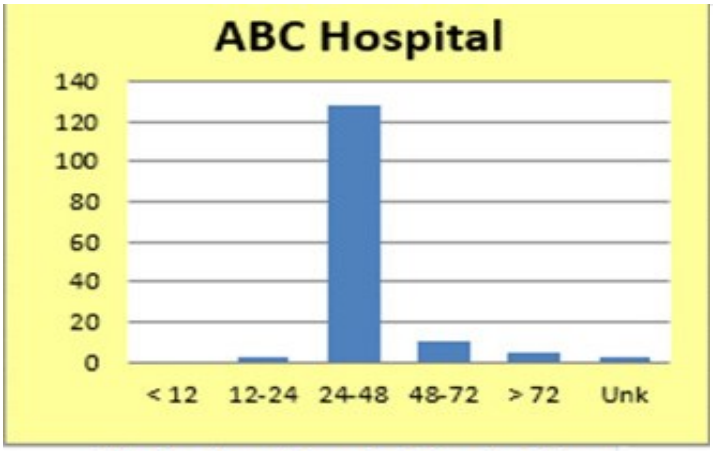
NewSTEPs data queries — *from page 6*

accuracy. Users can organize the information in a manner that makes sense and is useful for program tracking purposes. The goal is to have 90 percent of specimens collected and data identified within 48 hours. Initial efforts included queries for time to collection (time elapsed from birth to specimen collection); and time to receipt (time elapsed from sample collection to receipt in the laboratory). This was expanded to include receipt to report (time elapsed from receipt in laboratory to final report), birth to report (time elapsed from birth to final report); unsatisfactory specimens (number of specimens that are unsatisfactory for testing, sorted by collection and transport categories); and missing essential demographics (number of fields missing that are critical to testing and monitoring, i.e. time of birth). Each of these queries addresses the ACHDNC's timeliness recommendations in some way.

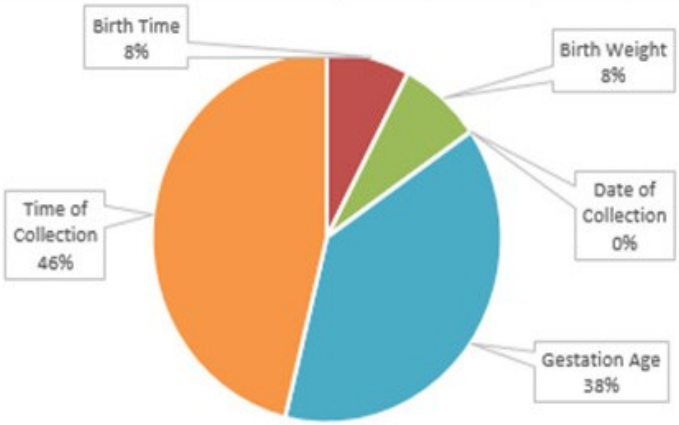
A valuable offshoot from these efforts is the ability to collect data about hospital-specific performance. The sample data shown in Figure 3 is fictitious, yet demonstrates areas where performance excels and improvements can be made. The newborn screening program staff, who maintain frontline communication with partners in Delaware hospitals and birthing centers, cover this information during their quality visits. This has proven to be a useful tool, well received by nurse managers.

Delaware is proud to be an active partner in this national collaboration. We expanded our reporting to include timeliness parameters involving time-critical disorders to prevent poor health outcomes in newborns.

Figure 3. Sample data: Where Performance Excels and Where Improvements Can Be Made



Distribution of Sample Collection Times



AGE AT INITIAL COLLECTION	Percent of samples collected within recommended 24-48 hours (goal 95%)			
	OCT	NOV	DEC	TOTAL
# Collected 24-48 hours	40	43	45	128
% Collected 24-48 hours	80.0%	82.7%	88.2%	83.7%
24-48 hours - State %	83.6%	84.0%	86.4%	84.7%

UNSAT SPECIMENS	2016			Period Total
	OCT	NOV	DEC	
# Unsatisfactory Samples	1	0	1	2
Percent of Total Samples	1.02%	0.00%	1.02%	0.68%
State Percent of Unsats	0.26%	0.16%	0.30%	0.24%

MISSING ESSENTIAL INFORMATION				
Reflect 1st & Repeats	OCT	NOV	DEC	TOTAL
Birth Date	0	0	0	0
Birth Time	0	1	0	1
Birth Weight	0	1	0	1
Date of Collection	0	0	0	0
Gestation Age	2	1	2	5
Time of Collection	2	1	3	6
TOTAL	4	4	5	13
Percent of TOTAL Spec	4.1%	4.1%	5.1%	4.4%
State Specific Percent	0.0%	4.7%	5.7%	3.4%

Employee News

DPHL welcomes **Curtis Harris, MD** as its CLIA Director. Dr. Harris received his Doctorate of Medicine from Howard University, College of Medicine. His internal medicine residency was at the Long Island Jewish Medical Center. Dr. Harris also spent nine years in the U.S. Air Force as a lieutenant colonel in the U.S. Air Force Medical Corp. and has held fellowship positions in hematology and oncology at the Brown University Program in Medicine. He recently retired as a medical oncologist from the Annapolis Oncology Center and Chesapeake Bay Oncology. Previously, he was the associate clinical professor of medicine at the University of Maryland School of Medicine. He has held the roles of CEO, medical director, and CLIA laboratory director at Chesapeake Bay Oncology. We are happy to have Dr. Harris

Indigo Johnson joined DPHL in February 2017. She currently holds the position of assistant laboratory information management system (LIMS) administrator where she is learning to trouble-shoot and resolve LIMS application issues. Originally from Allentown, Pennsylvania, she relocated to Dover in January 2012 with her cat Chen (pronounced 'shen'). A recent graduate from Delaware State University, she majored in biological sciences. Indigo has a passion for biomedical research with over two years of research experience focused on neurodegenerative diseases. She believes in making a positive impact in the communities of Delaware which is why she joined DPHL. In the future, Indigo plans to continue her education in biomedical research while continuing community service.

Rebecca Sahraoui works in the molecular virology section. She graduated with her Master of Science in molecular genetics from the University of Delaware in May 2016 and spent her graduate career at A.I. DuPont Hospital working on rare genetic disorders. She is excited to work at DPHL and has enjoyed every minute she has been with the lab.

Beth Clifton grew up in the Rockville, Maryland area and relocated to Delaware in 2009 shortly after graduating from Salisbury University with her bachelor's in biology. This was about one year after claiming that she would "never, ever – under any circumstances — live in Delaware." She spent the past six years working at DuPont, primarily focused on evaluating gene editing technologies in soy beans. Although she is still interested in gene editing, her current primary interest is in medical herbalism and she has completed one year of an herbalism program. In her spare time, Beth keeps busy by going to as many auctions as she can and currently has an antique booth in Bridgeville. Beth lives in Smyrna with her husband and son.

We would like to congratulate **Ben Voshell** in his new position of Microbiologist II at DPHL Microbiology Laboratory. Ben joined the Marine Corps and deployed to Iraq, Haiti, and Afghanistan. After receiving an honorable discharge, Ben attended Chestnut Hill College in Philadelphia and received his Bachelor of Science in molecular biology. In his free time, Ben enjoys spending time with his Labrador, Louie. Ben once scored four touchdowns in a single game at Polk High. His hobbies include Bigfoot hunting,

DPHL would like to congratulate **Lori Bellotti** in her new position of Laboratory Technician III at DPHL. 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, and 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.