Delaware LabOrator

Published by the Delaware Public Health Laboratory

Delaware Food Outbreaks 2018

by Gregory Hovan, B.S., M.B.A., Laboratory Manager, Delaware Public Health Laboratory

DELAWARE, ALONG WITH the rest of the United States, saw a dramatic increase in foodborne outbreaks in 2018. The Centers for Disease Control and Prevention (CDC) reported 24 multi-state food investigations in 2018[1], more than a two-fold increase over previous years. There have been direct associations with several of these national outbreaks from Delawareans. The Delaware Public Health Laboratory (DPHL) performs much of this significant investigational work in conjunction with the Office of Infectious Disease Epidemiology (OIDE).

Commonly associated foodborne pathogens, like *Salmonella*, *E. coli*, and *Listeria* cause severe illness, including gastroenteritis. In some cases, extremely severe infections, including bacteremia, hemolytic uremic syndrome (HUS) from *E. coli*[2], or invasive infections in pregnant women from Listeria[3], affect normally healthy individuals. These diseases can result in loss of kidney function or even death.

Many of these associated outbreaks are becoming commonplace, including the outbreaks of *E. coli* serotype O157 in lettuce and *Salmonella* serotype Newport in ground meat. These two outbreaks accounted for 385 cases and 110 hospitalizations. Foodborne illnesses profoundly impact public health through increased health care needs, and economically to the U.S. food supply.

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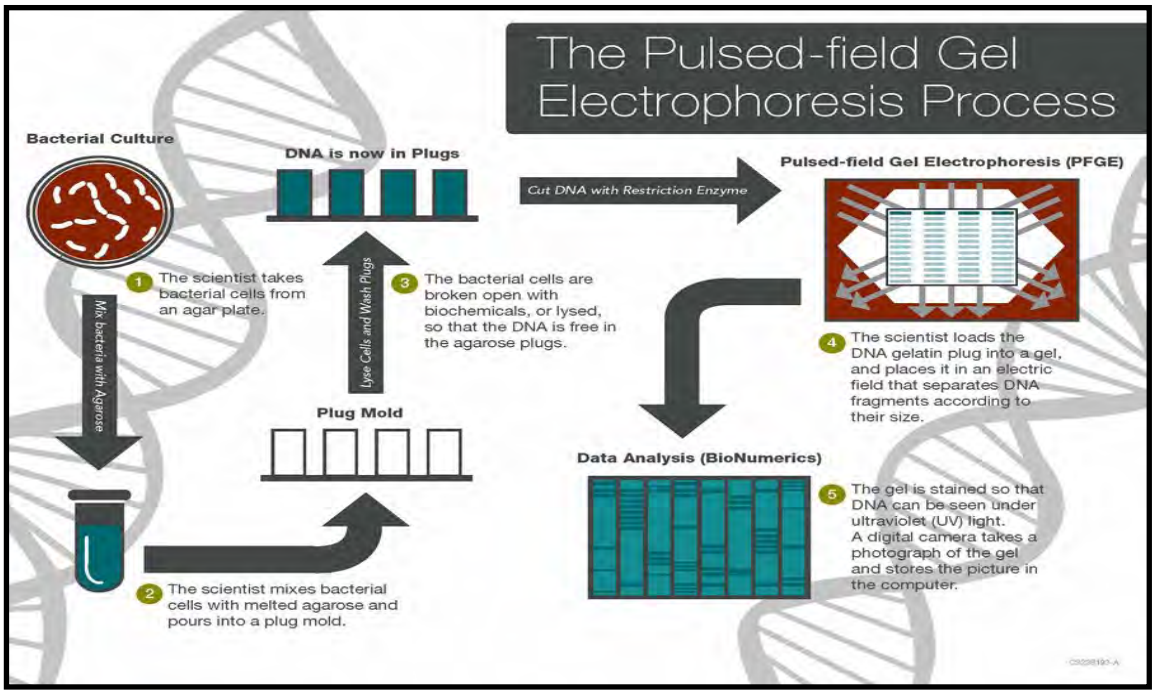
Figure 1. The PFGE Process



Source: Centers for Disease Control and Prevention

Spring 2019

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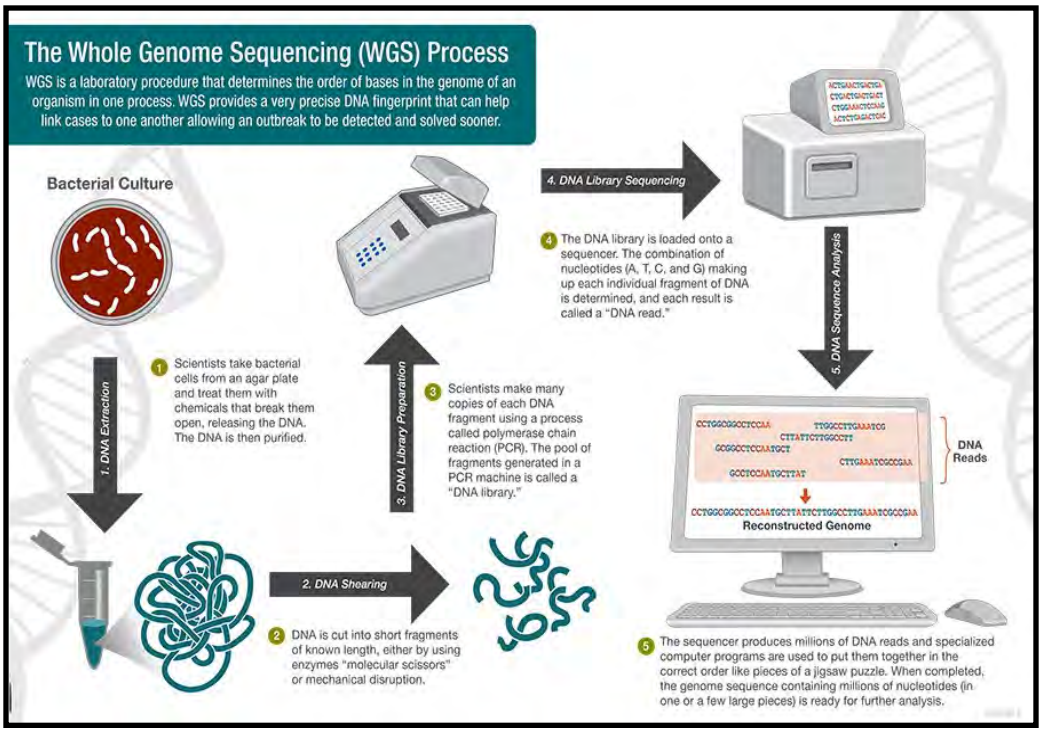
Increased sensitivity of the detection methods for foodborne illnesses at the CDC and public health laboratories were instrumental in 2018. For 20 years, Pulsed Field Gel Electrophoresis was the "gold standard" for identifying clusters and outbreaks related to foodborne pathogens. This method (Figure 1) uses fragments of DNA that are separated into bands, known as "fingerprints," by enzymes that cut the DNA in specific locations. By comparing these fingerprints to others, a pattern number is assigned. The pattern numbers allow scientists to associate these pathogenic strains across state lines, from food or human samples, to one another. One conundrum is that many serotypes of *Salmonella* are similar but genetically diverse (i.e. *Salmonella* enteritidis). This serotype will produce a very common pattern number despite its great genetic diversity. This makes distinguishing outbreaks difficult because they may produce the same PFGE pattern, even though they are unrelated.

Figure 2. The Whole Genome Sequencing (WGS) Process



Source: Centers for Disease Control and Prevention

With a full transition in 2019, CDC is implementing Next Generation Sequencing technology for Whole Genome Sequencing (WGS) of the pathogen's genome. These sequences represent all the letters of genetic code that make each organism unique. WGS is much more accurate to identify relationships between organisms.

This technology has already proven valuable in Delaware by identifying samples related to numerous outbreaks, including *Salmonella* in Honey Smacks™ cereal, Kratom, and clam chowder. DPHL and OIDE used WGS to relate Delaware *Vibrio parahaemolyticus* cases to Venezuelan crabmeat. This technology also provides results for antibiotic drug resistance, specifically to samples of *Salmonella* serotype Infantis found in raw chicken. DPHL and OIDE work closely to investigate common causes for these outbreaks and determine their relation. This includes daily emails and telephone calls. Last year was DPHL's second full year performing WGS on all foodborne pathogens received for testing. DPHL discontinued PFGE and performs WGS in real-time as a preferable method for tracking and identifying outbreaks. DPHL stays on the cutting edge!

DPHL shares all data generated from WGS with the CDC and its public health laboratory partners. It is also publicly available through a partnership with the National Center for Biotechnology Information (NCBI) and includes data from foodborne pathogens all over the world. All data-sharing conforms to federal law of client confidentiality through the Health Insurance Portability and Accountability Act and contains no personally identifying information. This encourages resource sharing between scientists, benefits communication, and supports public health efforts. All patients who suspect they have unresolved foodborne illnesses should be encouraged to see their physician and provide a specimen. Typing the specimen by WGS will prevent other infections and limit the spread of outbreaks.

References:

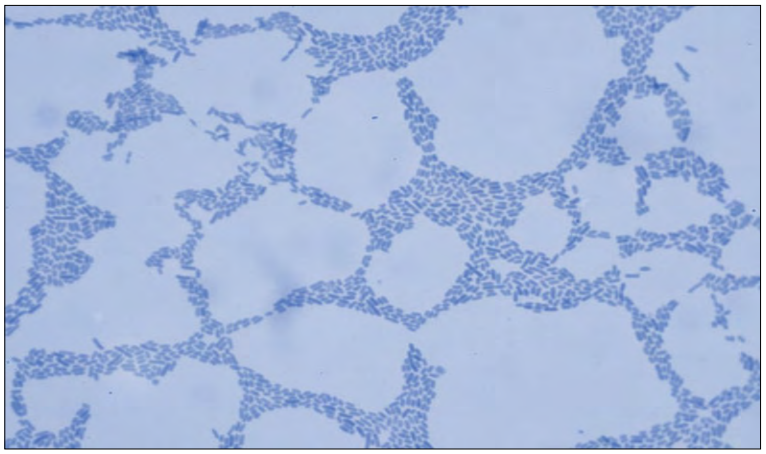
1. *List of Selected Multistate Foodborne Outbreak Investigations*, 2019, CDC: https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html
2. *E. coli* (Escherichia coli), CDC: https://www.cdc.gov/ecoli/ecoli-symptoms.html
3. *Listeria (Listeriosis)*, CDC: https://www.cdc.gov/listeria/symptoms.html

Playing in the Sandbox With Agents of Bioterrorism: A Review of the 2018 Wet Workshop for the Sentinel Laboratorians

by Becca Savage, BT Microbiologist II

On October 3 and 4, 2018, 19 individuals converged at DPHL to begin or continue their education on agents of bioterrorism. Participants represented many of the sentinel hospital laboratories, including Nanticoke, Christina Care, A.I. duPont, Bayhealth-Kent General, DPHL, and the Department of Agriculture. Instructors were Marion Fowler (Biosafety Official), Greg Hovan (Microbiology Manager I), Nancy Valeski (Microbiologist III), and Becca Savage (Microbiologist II), all of DPHL. Two groups allowed for a thorough, in-depth, and hands-on learning experience through presentations and wet laboratory demonstrations.

The day started with a pre-test to gauge the starting knowledge. The average test score was 59 percent. Nancy Valeski presented the history of bioterrorism. Attendees learned that crude bioterrorism occurred as early as 600 BC with rye ergot poisoning, and continued through modern warfare and the present time as seen with the U.S. anthrax investigations in 2001. All DPHL instructors shared an overview of bioterrorism agents, the role of sentinel and reference laboratories, and bioterrorism organisms. Each organism's disease transmission, clinical presentation, microbiological growth patterns, and biochemical characteristics were discussed in order for sentinel laboratory personnel to readily recognize these organisms. How sentinel laboratories should react when a bioterrorism agent is suspected was also presented. Morning presentations were rounded out with a biosafety and biosecurity presentation by Marion Fowler. She discussed laboratory-acquired infections and the importance of maintaining proper biosafety practices, especially when working with highly infectious organisms such as bioterrorism agents.



The favorite part of the day was the afternoon hands-on laboratory session. Each bioterrorism agent — *Bacillus anthracis*, *Brucella* spp., *Burkholderia mallei* and *pseudomallei*, *Francisella tularensis*, and *Yersinia pestis* — was assigned a station that included both "look alike" organisms and attenuated/vaccine organism strains. Participants circulated to each station with the help of the DPHL staff and recorded the growth morphology on various microbiological media 24, 48, and 72h of incubation for each organism, and the gram stain and biochemical characteristics of that same organism. The ability to view/study these look-alike and attenuated organisms helped the participants distinguish these agents from other organisms in a real-life scenario.

Attendees were then given the chance to test their first-hand knowledge to identify an "unknown" agent. For example, one station was a scenario in which a Minnesota farmer became sick after eating meat from a cow in his herd that was unable to rise. Participants had a short case description and recorded the growth morphology, gram stain, and biochemical results present at each station. Students made educated guesses as to what organism was the causative agent in each unknown scenario. The causative agents were unveiled in a group discussion at the end of the day. As almost every participant made correct conclusions, the DPHL instructors believed they had successfully taught the information. Finally, the participants took a post-test. The average score on the post-test was 86 percent — 27-point increase from that morning. DPHL staff were pleased to see such an improvement and were excited that we achieved our goal of helping the sentinel labs acquire improved knowledge on the bioterrorism agents. We enjoyed the opportunity to teach and help our health partners in the state, and look forward to teaching this workshop again in the future.

Molecular Virology Bids Goodbye to Viral Culture

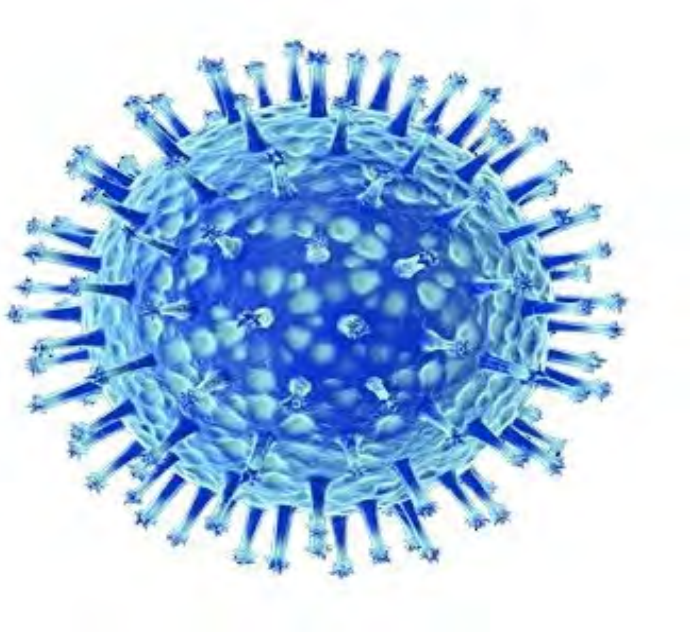
***by Emily Hanlin,* Laboratory Manager**

For a significant amount of time, virus isolation was the "gold standard" of diagnostic Virology (Hodinka & Kaiser, 2013). Early in its inception, the Virology Laboratory maintained cell lines in-house, including human epithelial cells (hep-2), Medical Research Council cell strain 5 (MRC-5), and monkey kidney epithelial cells (Vero) for the growth of respiratory viruses like influenza, herpes viruses like Varicella Zoster Virus, and enteroviruses like Coxsackie B. Maintaining healthy cell growth lines and passage of cell lines from one growth medium to another required extremely stringent aseptic technique and significant technical precision.

As technology progressed, the lab purchased tubes affixed with the various cell lines. Tubes were inoculated with samples and observed under the microscope for cell death, also known as Cytopathic Effect (CPE). Once cell death was noted, immunofluorescent antibody tests identified the viral agent present. However, virus isolation was not without its own set of issues. Tubes and subsequent shell vial culture (centrifugation onto a small cell monolayer to increase virus infectivity), required weekly estimation of samples (Navarro-Mari, Sanbonmatsu-Gomez, Perez-Ruiz, & De La Rosa-Fraile, 1999). The lab discarded tubes and vials often, because the estimates from the weeks and months before had decreased. Sometimes, there were not enough tubes or vials due to a heavier-than-usual respiratory season. Additionally, the presence of CPE does not guarantee confirmation of a viral infection since subsequent antibody testing was limited to a more narrow scope of certain viruses. Perhaps the most significant downside to viral culture was the amount of time it takes some viruses to grow, sometimes up to two weeks. Doctors depend on test results to help guide diagnoses, but in many cases with viral culture, patients had already recovered.

With the progression of multi-plex real-time Polymerase Chain Reaction (rPCR) assays, moving respiratory viral pathogen tests, including influenza, required very little debate. Molecular methods are sensitive and specific, but more importantly, fast. Methodologies can be performed in as little as two hours. Given this, molecular results are used to guide treatment decisions (Hodinka & Kaiser, 2013). Moreover, DPHL uses the CDC's influenza rPCR, which uses primer and probe sequences expressed by all subtypes of influenza A, with specific subsequent rPCR methods for the most predominant types. This allows the lab to detect any potential novel influenza A viruses.

DPHL uses the GenMark eSensor Respiratory Virus Panel for the detection of other respiratory viruses, including RSV A and B, Parainfluenza 1, 2, and 3, Human Metapneumovirus, Rhinovirus, and Adenovirus B, C, and E. The final viral culture method to transition to



molecular was Herpes Simplex Virus 1 and 2. DPHL transitioned to Quidel's Lyra Direct HSV 1+2/VZV assay on January 2, 2019. The laboratory included VZV (chickenpox) using this method, because it is very slow and laborious to isolate via culture. DPHL lost Enterovirus capabilities; however, most reference laboratories and CDC can provide Enterovirus rPCR going forward.

While molecular assays may be more costly, there will be less waste with estimating the number of weekly tubes for culture. Unfortunately, like virus isolation, rPCR is limited to known viruses, since it is dependent on complementary sequences. It is only a matter of time before virus detection progresses even further into Next Generation Sequencing.

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Navarro-Mari, J.M., Sanbonmatsu-Gamez, S., Perez-Ruiz, M., De La Rosa-Fraile, M. (1999). Rapid Detection of Respiratory Viruses by Shell Vial Assay Using Simultaneous Culture of HEp-2, LLC-MK2, and MDCK Cells in a Single Vial. *Journal of Clinical Microbiology. July 37(7):* 2346-2347. Retrieved from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC85156/

LPAC Fall 2018 Meeting Update

by Margaret J. Zimmerman, Lab Manager, Delaware Public Health Laboratory

THE FALL MEETING of the Delaware Laboratory Preparedness Advisory Committee (LPAC) was held at the Delaware Public Health Laboratory (DPHL) on September 25, 2018. A wide range of topics were presented, ranging from presentations by the Office of Animal Welfare (OAW) and the Department of Agriculture (DDA) to an update on what might be expected from the 2018-2019 influenza season.

The meeting started with a presentation from Christina Motoyoshi, OAW Director. Ms. Motoyoshi shared facts about households with at least one pet and the benefits of the Human-animal bond. The OAW mission is "… committed to protecting the health, safety, and welfare of companion animals, and dedicated to promoting the human-animal bond in the state of Delaware." OAW fulfills its mission by protecting animals from abuse and neglect and supporting programs that reduce pet homelessness and cruelty. Ms. Motoyoshi spoke about the low-cost spay and neuter clinics and an animal welfare license plate that is available through the Delaware Division of Motor Vehicles. A portion of the one-time plate fee goes towards sheltering homeless animals and providing spay and neutering finances to low-income pet owners. Contact information for Delaware Animal Services, the enforcement branch of OAW, was provided along with contact information for Brandywine Valley SPCA, their sheltering partner. Ms. Motoyoshi shared success stories about rescued animals.

Dr. Heather Hirst, Doctor of Veterinary Medicine with DDA, shared "Goat Yoga and Other Related Animal Fun." The Goat Yoga movement began in Oregon in 2016. Participants perform yoga poses with goats. These goats interact by sitting on or near humans while they are in yoga poses. It is therapeutic and relaxing to interact with an animal on this level. Dr. Hirst explained that Animal Assisted Therapy, according to a published study, decreased stress hormones such as cortisol, adrenaline, and aldosterone and increased "health-inducing and social-inducing" hormones such as oxytocin, dopamine, and endorphins after 20 minutes with a therapy dog.

Dr. Hirst detailed her job activities. Since the State of Delaware does not have a public health veterinarian, the DDA is always willing to assist on cases that involve exposure to animals that may have carried or transmitted disease. There is more data on poultry in the urban setting because more people have backyard chickens. She also discussed emerging tick-borne illnesses.

Emily Hanlin, laboratory manger of the DPHL Virology section, briefed the audience on the 2018-2019 influenza season that started October 1, 2018. Ms. Hanlin went over the DPHL/CDC protocols for surveillance testing of influenza. She also discussed DPHL adding Polymerase Chain Reaction testing, which identifies the rabies virus.

Rebecca Savage, a microbiologist with the DPHL Microbiology section, provided updates on the LPX exercise; an upcoming Wet Workshop which took place on October 3 and 4, 2018; and man-down training in DPHL.

The last presentation of the day was from Greg Hovan, laboratory manager of the DPHL Microbiology section. Mr. Hovan updated the group on the progress of surveying and cataloging Carbapenem Resistant Organisms in Delaware. DPHL received and reported 113 new isolates since the spring 2018 LPAC meeting. Mr. Hovan also reviewed the progress of whole genome sequencing in identifying and cataloging sources of enteric outbreaks.

The meeting concluded with a roundtable, during which attendees could share news.

Employee News

Welcome, Lacey!

Lacey Hall. DPHL welcomes Lacey as the new Lab Tech III for the Environmental Chemistry Laboratory. Lacey is from right outside Philadelphia and recently moved to Delaware to take this new position. She graduated from Villanova University in May 2018 with a B.S. in Environmental Science and hopes to return for her Masters. Lacey enjoys camping, going to the shore, and spending time with her family and pets.

Welcome, Taylor!

Taylor Moore. We welcome Taylor as our new Contractual Chemist in the Environmental Chemistry Laboratory. Taylor received her B.S. Degree in Biochemistry from the University of Delaware. While in college, Taylor worked as a Formulation Scientist at ACT Solutions, where she created various personal care products (shampoos, conditioners, deodorants, etc.). In her free time, Taylor enjoys spending time with her family, doing do-it-yourself projects, and playing with her dogs.

Welcome, Allison!

Allison Naumann. DPHL welcomes Allison as our new Contractual Laboratory Scientist in the Virology section. Allison recently graduated from the University of Delaware with a Bachelors of Biomedical Engineering. As a senior at the University of Delaware, Allison worked with Merck & Co. to create a new tool that efficiently aids in ongoing developments for the Measles, Mumps, & Rubella vaccine. In her free time, she enjoys playing video games and attending comic conventions.

Welcome, Karen!

Karen Pollard. Join us in welcoming Karen to DPHL as our new Laboratory Manager I. Karen has an Associate of Health Sciences degree in Medical Laboratory Technology, a B.S. in Biology with a minor in Chemistry, and a Master of Science in Health Care Management. She has over 20 years of experience working in clinical and educational laboratory settings, with a primary concentration in Microbiology. Karen has a husband James, an 8-year-old son Robert, a 5-year-old-son Jacob, and a Boxer named Penny. In her free time, she enjoys spending time with her family, crocheting, and traveling.

Congratulations, Randy!

Randy Correia. Randy has accepted a new position in the laboratory as an Environmental Control Technician II. Since August of 2016 he was a Supply, Storage and Distribution Technician I in the DPH warehouse. Prior to joining DPHL, Randy worked for Wal-Mart as a Regional Compliance Manager and he retired from the Air Force Reserve after 30 years of service. He has a Master of Science degree in Human Resource Management from Wilmington University. Randy has a wife and three children.