In This Issue

The National Molecular Subtyping Network for Foodborne Disease Surveillance

PulseNet™ News

State & Local Public Health Laboratories in the United States and PulseNet Canada

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BioNumerics Software Workshops in Atlanta, GA

Molly Joyner, Centers for Disease Control and Prevention, Atlanta, GA

Two BioNumerics Software Workshops were conducted August 22 – 26, 2005 in Atlanta, GA. Workshop participants were divided into two groups and each group attended a two-day session during the week: Group 1 consisted of 19 people from 14 laboratories and Group 2 consisted of 19 people from 18 laboratories. The workshops combined beginning and intermediate features of the software and PulseNet customizations, so the participants were able to learn everything from the basic installation process to advanced querying and data management techniques.

The workshop included an overview of BioNumerics and the PulseNet Master Scripts, steps to analyze a TIFF image, methods to enter and link data to a corresponding gel, and how to upload to the online PulseNet databases. Participants also learned ways to perform comparisons and queries in their local databases as well as the national database. More advanced features of the training sessions included importing data from Microsoft Excel spreadsheets into BioNumerics, performing advanced queries, and working with subsets, unique pattern lists, and composite datasets. Also, PulseNet bundle files and communication via WebBoard were covered in both sessions.

"This course has taken me well into the PulseNet environment. Without it, BioNumerics is an incredibly tall mountain to scale."

Participants performed practice exercises to reinforce the concepts they were learning. Local databases were set up for each participant which simulated individual labs. Participants were able to practice uploading to the national server, and downloading information from the national server to their local databases.

Participant evaluations and comments indicated that the workshops were extremely helpful. All participants said they would recommend this course to other public health laboratories. One person mentioned, "This course has taken me well into the PulseNet environment. Without it, BioNumerics is an incredibly tall mountain to scale." CDC

Bionumerics Workshop for Pulsenet Participants
August 22-26

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PulseNet Lab Workshops in Atlanta, GA

Kara Cooper and Mary Ann Fair, Centers for Disease Control and Prevention, Atlanta, GA

Two consecutive workshops for the "Standardized Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-Field Gel Electrophoresis" were conducted the week of August 22-26, 2005 by PulseNet USA, CDC, and APHL. Since the new training facilities at CDC were not available, the PFGE training was held at the Georgia Public Health Laboratory in Atlanta, GA. Twenty-one participants, representing health departments from two counties, 17 states and the District of Columbia (DC) and one FDA laboratory were divided into two groups with each group completing the training in the standardized PulseNet PFGE protocol for Salmonella in two and half days.

The workshop used seven Salmonella strains to provide instruction, demonstrations, and hands-on experience in PFGE from plug prepara-

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Lab Workshops in Atlanta, GA

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tion to staining and imaging the gel. The resulting gels were imaged using the Bio-Rad Gel Doc XR imaging system; the participants were shown how to convert the very large image files obtained on the Gel Doc XR to smaller TIFF files that can be analyzed with the BioNumerics software (Refer to WebBoard Conference "Image Acquisition" Topic "Gel Doc XR"). Additionally, the participants were trained in the operation and maintenance of both CHEF Mapper and DR-III electrophoresis systems.

In addition to the laboratory experience, CDC and BioRad staff presented lectures on the goals of PulseNet, the PulseNet standardized PFGE protocols for foodborne pathogens, the principles of PFGE, molecular epidemiology, and NARMS, and FoodNet. Additional presentations were given on safety issues in molecular subtyping by PFGE, troubleshooting common problems with PFGE, the current status of MLVA protocols, and standardized PFGE protocols for several non-foodborne pathogens.

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The trainee evaluations gave the laboratory workshop an overall rating of excellent. Most commented that the "hands on" laboratory experience was the most helpful activity in the workshop and that it would positively influence their ability to perform PFGE of enteric and other pathogens. The workshop training staff thanks all the students for making this workshop a successful and enjoyable learning experience for all involved.

CDC

Mountain Area PulseNet Conference

Jenni Wagner, Utah Public Health Laboratory, Salt Lake City, UT

The Mountain Area held its very first PulseNet conference April 5 and 6, 2005 in Salt Lake City, Utah. Thirteen PulseNet participants from four states—Arizona, Colorado, Utah, and Wyoming—attended, along with several people from Minnesota Department of Health, CDC, APHL, and the National Laboratory Training Network (NLTN). Attendees included laboratorians, epidemiologists, and lab directors.

The states included in the Mountain Area are very diverse in their PulseNet activities. Some of the states are involved with their state veterinary laboratories and run Pulsed-Field Gel Electrophoresis (PFGE) on many of the veterinary isolates. Other states are involved with their local hospitals in performing PFGE on nosocomial outbreak isolates. Other states are involved with their local hospitals in performing PFGE on nosocomial outbreak isolates. Many of the Mountain Area's PulseNet participants have other job duties including bacteriology, bioterrorism preparedness, and other molecular techniques which can pull them away from doing PFGE and their CHEF Mappers frequently. The yearly number of isolates subtyped by PFGE within the region ranges from a couple of hundred for some labs to over one thousand samples for others.

> Focus was on communication and identifying areas in which participants could make improvements.

The goals of the Mountain Area meeting were similar to the other three PulseNet area meetings, with a focus on communication and identifying areas in which participants could make improvements. During the brainstorming session, states came up with ideas to unify and advance PulseNet-related activities in their respective states, as well as in the area labs. States identified many

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Some of the meeting attendees enjoying a group dinner

Mountain Area Regional Meeting

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common concerns such as limited funding poor epidemiology/laboratory interactions, limited crosstraining, and poor sample collection compliance.

States proposed many solutions, with some being put into action on the spot. Some states are setting up regular meetings with their laboratorians and epidemiologists to facilitate a cooperative relationship. To better meet real-time PFGE expectations, cross-training of employees has become a priority in some states. Outbreak investigation kits are being assembled, allowing patients to mail their stool samples to the public health labs to help alleviate some of the compliance issues. These were just a few of the several plans that will help attendees reach the goals set during this meeting. Reasonable deadlines were given to each state to help them attain these goals in a timely manner. Progress on each goal will be followed with quarterly area conference calls.

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The meeting attendees filled out evaluation forms at the end of the conference. Their responses indicated that this meeting was worthwhile. Several attendees agreed that this meeting excelled in actively approaching difficult issues and actually following through with realistic solutions.

The PulseNet Mountain Area is a unique group of states, each with a different focus. These differences mean that states rely on each other when the unexpected occurs. The relationships formed through this collaboration help to build a strong and pro-active PulseNet area. Participants in the PulseNet Mountain Area are excited about the next area meeting if only to maintain successful working relationships with a great group of people! CDC

ANNOUNCEMENTS

- **The 2005 PulseNet Update Meeting and 2005 National Foodborne Epidemiologists Meeting presentations are available on the Association of Public Health Laboratories website: http://aphl.org/conferences/pulsenet_update_meeting_2005/.**

PUBLICATIONS

- Gerner-Smidt P, Kincaid J, Kubota K, Hise K, Hunter SB, Fair MA, Norton D, Woo-Ming A, Kurzynski T, Sotir MJ, Head M, Holt K, Swaminathan B. **Molecular Surveillance of Shiga toxigenic** *Escherichia coli* O157 by PulseNet USA. J Food Prot. 2005 Sep; 68(9):1926-1931.

- ***Shigella flexneri* Serotype 3 Infections Among Men Who Have Sex with Men** --- Chicago, Illinois, 2003-2004. MMWR. 2005 August, 26, 54(33); 820-822.

- Graves L, Hunter S, Ong A, Schoonmaker-Bopp D, Hise K, Kornstein L, DeWitt W, Hayes P, Dunne E, Mead P, Swaminathan B. **Microbiological Aspects of the Investigation That Traced the 1998 Outbreak of Listeriosis in the United States to Contaminated Hot Dogs and Establishment of Molecular Subtyping-Based Surveillance for *Listeria* monocytogenes in the PulseNet Network.** J of Clin Microbiol, 2005 May, 43 (5): 2350-2355.

ARIZONA STATE PUBLIC HEALTH LABORATORY

Graham Briggs and Stephanie Kreis, Arizona State Public Health Laboratory, Phoenix, AZ

The Arizona State Public Health Laboratory (ASPHL) has recently moved into a new, state-of-the-art facility in Phoenix. This new facility has enabled ASPHL to house new equipment and provide additional testing. The new laboratory is three times larger in area when compared with the older facility. Also, the ASPHL has increased its ability to work with dangerous pathogens by increasing the number of BSL3 suites from two to five. The addition of a training laboratory allows the ASPHL to provide seminars and training for local and regional laboratory staff including recent National Laboratory Training Network (NLTN) training courses on blood-borne parasitology and identification of organisms in stool samples sent for Ova and Parasite examinations (O&P's).

The ASPHL is comprised of numerous sections including bacteriology, virology, environmental microbiology, serology, bio-emergency detection and response, newborn screening, numerous chemistry sections, and a laboratory licensing division.

A number of new assays are currently being developed, evaluated, and implemented in a variety of areas. Examples include the introduction of mass spectrometry for newborn screening, gas chromatography as an additional method for bacterial identification, and automated DNA sequencing for pathogen identification and characterization. Current projects utilizing sequencing include molecular characterization of methicillin-resistant Staphylococcus aureus (MRSA), Coccidioides species, and Norovirus isolates for epidemiological investigations. An EID fellowassigned to Arizona is now working with both the laboratory and epidemiology staff to better characterize an illness unique to the southwest, coccidioidomycosis, using sequencing and molecular techniques. In addition, the ASPHL has been selected by CDC and the Association of Public Health Laboratories as one of the sites to validate the second generation sequencing based PulseNet subtyping method for E. coli O157:H7, Multi-Locus-Variable Number of Tandem Repeats Analysis (MLVA). This method may supplement PFGE for E. coli O157:H7, depending on results from a number of laboratories performing the validation. Finally, the Arizona Department of Health Services has been working to foster relationships with academia by welcoming interns, developing projects with academic faculty, and using students during outbreaks to increase surge capacity for investigations.

View of AZ State Public Health Laboratory

Stephanie Kreis in AZ State Public Health Laboratory

All PulseNet and NARMS related activities are performed in the ASPHL's bacteriology unit. Arizona currently has one full-time employee devoted to PFGE and is in the process of hiring a second full-time employee who will provide additional help with PFGE. Arizona routinely performs PFGE on every Salmonella, Shigella, Listeria, and Shiga-toxin producing E. coli isolate received. Also, Arizona will begin subtyping all Yersinia pestis isolates after recieving training at the CDC in Fort Collins, CO in the near future. The ASPHL also uses PFGE to subtype other organisms such as Bordetella pertussis, Acinetobacter, Pseudomonas, Group A Streptococcus, and Vancomycin Resistant Enterococcus (VRE) upon request from our epidemiology section.

PFGE has become an essential part of many epidemiological investigations in Arizona, particularly involving routine enteric organisms. The ability to communicate with other states and the CDC through PulseNet has allowed Arizona to identify numerous isolates involved in outbreaks that would have otherwise gone unnoticed. For example, Arizona, like many other states, identified cases of Salmonella infection linked to almond consumption last year, but only after Oregon had identified the PFGE subtype and source of exposure. This allowed Arizona to search for the "outbreak" pattern at the local level, interview suspect cases, and identify whether Arizona cases had exposure to the same product as Oregon cases.

PFGE also allows the ASPHL to identify disease clusters of even highly clonal serotypes such as Salmonella Enteritidis (SE). The ASPHL has identified numerous SE outbreaks in recent years related to travel to Mexico using PFGE as one of the primary investigative tools. In Arizona, there are couple of dominant XbaI patterns of SE, one of which is usually associated with travel to Mexico. The ASPHL lab has been able to identify this pattern from groups of college students

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MOU Meeting
PulseNet USA and PulseNet Canada

Memorandum of Understanding Signed Between PulseNet USA and PulseNet Canada

Lai King Ng, National Laboratory for Enteric Pathogens, Winnipeg, Canada

PulseNet USA and PulseNet Canada are virtual laboratory networks with central national databases housed at the Centers for Disease Control and Prevention (CDC) in Atlanta, and the Public Health Agency for Canada's National Microbiology Laboratory (NML) in Winnipeg. On August 12, 2005, a Memorandum of Understanding (MOU) was signed by Canada's Health Minister and a United States Ambassador to enable the connection of servers containing the databases of PulseNet USA and PulseNet Canada. The connection of both databases through this MOU permits real-time data sharing and comparisons of genetic fingerprints of bacteria as they are generated in different laboratories across North America. The increase in foreign travel and rapid dissemination of food globally makes North American citizens more vulnerable to the threat of emerging and re-emerging infectious disease outbreaks and potential bioterrorism. Together, CDC and NML will continue to improve the ability to rapidly identify infectious agents and clusters of diseases. In particular, CDC and NML will use this MOU as a model to establish collaboration with other countries ensuring timely international public health response to mitigate the impact of natural and intentional foodborne illnesses. CDC

MOU Signing in Winnipeg, Canada

Laboratory Profile: Arizona

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who acquire this subtype of SE while vacationing in the same place in Mexico during spring break. After returning to Arizona, they seek medical attention from providers in each of Arizona's major college towns.

PFGE has demonstrated its usefulness with non-enteric organisms as well, albeit in a more limited fashion. PFGE is used numerous times a year to investigate nosocomial outbreaks of organisms like Pseudomonas, Serratia, MRSA, and most recently a cluster of invasive Group A Streptococcus (GAS) cases. This time PFGE allowed for the quick subtyping of a particularly aggressive strain of GAS that appeared to be spreading in a hospital. The pattern generated was then compared to isolates obtained after swabbing nearly 700 contacts of the first cases. A number of contacts were identified with the outbreak strain and given prophylactic antibiotics. This pattern was also identified when historical isolates that were associated with invasive disease were tested and compared by PFGE. The ability to subtype outbreak organisms using PFGE has greatly augmented ASPHL's ability to detect and investigate clusters of disease in Arizona. As everyone knows, the promise of next generation subtyping using genetic sequencing is just around the corner. CDC

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Welcomes:

- **Christopher Ball** has assumed the PFGE and analysis responsibilities at the Idaho Bureau of Labs, after transferring from a BT position within the lab. Chris also continues to develop and support molecular diagnostic Cactivities.
- Missouri Department of Health welcomes **Stephanie Barnhart**, a Public Health Laboratory Scientist, into the Microbiology Unit of the MO SPHL.
- **Robin Broeker** joined the *Listeria* Reference Laboratory at CDC in September 2005. Robin graduated from the University of Georgia with a Master of Science in Food Science and Technology. She will work with *Listeria* special projects, evaluate second-generation typing methods and provide support for the *Listeria* Reference Laboratory.
- California Department of Health Services' Enterics Laboratory welcomes **Samarpita Fontanoz** to the PFGE section. She is a recent graduate in Biology from San Francisco State University. We are also happy to have **Rachel Nieda** back after the birth of her son, Christopher.
- The WV Office of Laboratory Services would like to welcome **Megan King**, Microbiologist I, to the PulseNet team. Megan started in February 2005. She is a 2002 graduate of Ohio State University. Megan recently attended the PulseNet bench training and BioNumerics training in Atlanta the week of August 22-26. She will also be working in bacteriology, rabies, TB, and parasitology.
- **Tim Monson** has joined the Wisconsin State Laboratory of Hygiene (WSLH) PFGE team. He has worked as a microbiologist at WSLH for the past 15 years in the Bacteriology, Parasitology and Mycobacteriology Laboratories and is the WSLH Food and Waterborne Disease Program Coordinator. He is assuming many of the duties performed by **Terry Kurzynski**, a 9-year veteran of PulseNet, who retired at the end of June and is greatly missed. He will work closely with current WSLH PulseNet members Linda Machmueller and Simone Warrack and looks forward to working with everyone else involved with PulseNet as well.

Farewells:

- **Annette Malan** left the Idaho PFGE lab in June. She contributed greatly to the development of ID PFGE laboratory. We wish her only the best.
- **Mary Kate Cichon**, a valuable member of the PulseNet team, left the Massachusetts PFGE lab in August 2005. She will be missed here, but we wish her the best of luck in Colorado.

HOW WOULD YOU LIKE TO RECEIVE THE PULSENET NEWSLETTER?

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