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The National Molecular Subtyping Network  
for Foodborne Disease Surveillance



# PulseNet<sup>TM</sup> News

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



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## Proficiency Testing: Summary and Tips for Improvement

Jennifer Kincaid, Centers for Disease Control and  
Prevention, Atlanta, GA

*Salmonella*, *E. coli* O157:H7 and *Shigella* cultures were sent out in September for the fall 2006 round of PulseNet PT. Seventy-five certified laboratories participated in the *Salmonella* PT. Twenty-two laboratories submitted gels of excellent quality and 16 of those 22 received perfect scores. Sixty-two certified laboratories participated in the *E. coli* O157:H7 PT. Twenty-one laboratories submitted gels of excellent quality and fifteen of those 21 received perfect scores. Forty-four certified laboratories participated in the *Shigella* PT. Six laboratories submitted gels of excellent quality. For additional information on the results of the spring round of PT, please visit the WebBoard (Certification and PT conference) for a tabulated summary.

Some common mistakes and things to consider for the next round of PT and all routine submissions:

- Running the gel so that the last band of the standard is approximately 1.0-1.5 cm from the bottom of the gel (per PulseNet protocol) will help keep the images within the databases standardized and may improve the separation of bands. As noted in the protocols, you must optimize the run time based on conditions within your laboratory.
- If you are experiencing light bands, especially at the bottom of the gel,

repeat restriction with a larger plug slice, more units of enzyme for a longer amount of time, and/or with a different lot of enzyme. Wash the plugs at least two more times with TE Buffer to remove excess lysis reagents or other impurities before repeating the restriction. If you cannot see the bottom bands of the standard lanes, the gel should be repeated—this could indicate that you are also missing bands in the test lanes. You should also be sure that you are using the correct values for the cell suspension concentration. Another potential correction for light bands in the lower part of the gel is to adjust the turbidity, colorimeter or spectrophotometer readings so that the cell concentration is slightly higher.

- Zoom in during image capture. A gel image that fills the entire window on the imaging screen of the computer (without cutting off wells or the bottom of the gel) will make analysis easier and improve comparisons within the national databases.
- The camera in the Gel Doc XR has a much higher resolution than older Gel Doc systems, thus making the file size much larger (~1.2Mb compared to ~304 Kb). Although the larger image can be analyzed in BioNumerics, it is not ideal for file storage at CDC and the tripling of file sizes would not be manageable. The default settings that are set up when the PulseNet scripts are installed (thickness of image strips, resolution, etc.) are set for an image that is ~300-400 Kb in file size. After the image acquisition step is completed, the file size of the image can be reduced during the Export to TIFF step. For more information, please see the standard operating procedure "Image Acquisition and the Production of TIFF Files (PNL07)"

posted within the QA/QC Manual conference on WebBoard.

- After the lanes are defined in step one of analysis, adjust the "thickness" under "edit settings" to increase or decrease the thickness of the defined gel strips so that the left and right edges of the strips are just outside the edges of the bands. This will ensure that the bands will appear in a size appropriate for analysis. If the gel strips are defined incorrectly (too narrow), the edges of the bands will be cut off making it difficult to distinguish between a thick single band and a doublet.
- When deciding if a band is a doublet or a single band, look for white space in the middle of the area or indentations (i.e., doughnuts or shoulders) on the sides of the area that separate two bands. If indentations or white space are apparent on a printed copy of the TIFF, the area is most likely a doublet. If the indentations or white space are questionable, the area probably should be marked as a single band. Separate peaks on the densitometric curve may also provide information on the correct band designation of the area. **CDC**



### ANNOUNCEMENT:

Check out the updated Certification SOP (PNQ02) posted in the QA/QC Manual conference on WebBoard. Two changes have been made:

1. H9812 standard lanes are no longer included in analysis certification bundle files.
2. If an individual is having a difficult time becoming certified in gels and/or analysis, their Area Lab will be notified.



Austin, TX - May, 2006

## 2006 South Central Regional PulseNet Meeting

Ana María Valle-Rivera, Texas Department of State Health Services, Austin, TX

The South Central Region held its first meeting on May 18-19, 2006 in Austin, TX. The meeting was hosted by the Laboratory Services Section of the Texas Department of State Health Services (DSHS). Meeting participants arrived from New Mexico, Oklahoma, Louisiana, Arkansas, the city of Houston and DSHS. Also in attendance were members from Minnesota Department of Health, CDC, APHL and the National Laboratory Training Network (NLTN).

The main goal of the meeting was to improve the use of PFGE laboratory analysis as a tool in disease surveillance and epidemiological investigations by identifying and providing solutions to areas that need improvement.

Each participating group provided a brief presentation reviewing foodborne disease surveillance in their state or city. The presentations showed the unique situations encountered by each team and also provided alternate ways of performing our work.

There were three break-out sessions to brainstorm ideas to improve PulseNet activities. Some of the concerns identified include loss of funding, poor epi-lab interactions and non-submission of isolates to PulseNet. Some of the solutions suggested included taking your lab/epi to work day, looking for other grant opportunities, submission of joint lab/epi ELC applications, re-education of the county labs so they can communicate with clinical labs, and education of clinical/outside partners on how PulseNet data are used, among others.

Area conference calls will be arranged to discuss the progress on goals and solutions that were developed during these sessions.

Data from the evaluation forms filled by attendees indicate that the meeting was needed. Multiple attendees indicated they would like to have the meeting at least every two years to have the chance to interact with their area counterparts and exchange ideas. The host lab is very grateful to Minnesota Department of Health, CDC, APHL and NLTN for their assistance and guidance. 



East Lansing, MI - June 2006

## 2006 Midwest Regional PulseNet Meeting: Bridging the Gaps

Simone Warrack and Tim Monson, Wisconsin State Laboratory of Hygiene, Madison, WI

On June 15 and 16 of 2006, representatives of several Midwestern laboratories and health departments gathered at the Kellogg Center in East Lansing, Michigan for the Midwest PulseNet Regional Meeting. Epidemiologists and laboratory scientists came from Indiana, Kentucky, Michigan, Minnesota, Ohio, Wisconsin, and the Milwaukee City Health Department. The meeting was presented by the Michigan Department of Community Health (MDCH), PulseNet USA, and the Association of Public Health Laboratories.

Some of the conference goals and objectives included the identification of issues affecting foodborne illness detection, discussion of state and federal collaborations, and increasing the understanding of epidemiology and laboratory roles. Furthermore, strategies were discussed to increase the effectiveness of PulseNet. The meeting format, with presentations from each health department and subsequent discussions, provided an excellent opportunity to examine these issues.

After welcoming remarks by James Rudrik and Frances Pouch-Downes of the MDCH, John Besser of Minnesota spoke about the importance of PFGE and surveillance. Thai-An Nguyen of the CDC explained how epidemiologic investigations are handled at the federal level, the interaction with states, and how PulseNet data is used. Then, Kelley Hise, from the CDC gave an overview of PulseNet USA that explained the process of how PulseNet works and the three elements of PulseNet: data, data analysis, and data exchange.

Next, each health department presented a description of the overall process, both at the epidemiological and laboratory level. Breakout sessions were conducted to identify strengths, weaknesses, opportunities and threats to the goal of serving the public health. Each group consisted of an assortment of states and roles (epi and lab) so that many voices were represented. Some of the common concerns voiced by many groups included: obtaining isolates from clinical labs, specimen collection and the availability of collection kits at the local health department, information technology support for BioNumerics, staffing, funding, and data management. These working group discussions were summarized and presented to all attendees on day two. That afternoon, the health department attendees reunited to determine individual action plans. Each health department presented their plan to the entire group, allowing for feedback and the exchange of ideas. Some of frequently mentioned goals included improving communication at the local health

(Continued on page 3)

## 2006 Midwest Regional PulseNet Regional Meeting

(Continued from page 2)

department level, utilizing public relations, possible legislative action to improve isolate submission, taking advantage of federal grants, and face to face meetings between laboratorians and epidemiologists.

Day two also included presentations on several interesting topics: David Boxrud and John Besser from the Minnesota Department of Health spoke about common PFGE pattern interpretation issues. A presentation entitled "Linking Foodborne Surveillance Systems," which described the complexity of sharing data among PulseNet, eFORS, NARMS, FoodNet, etc. was presented by Kathryn Teates of the CDC. Next, Brad Deacon of the Michigan Department of Agriculture presented, "Agroterrorism, Food & Agricultural Security & Emergency Management." This talk highlighted the threats that the food and agriculture industry may face in light of accidental or deliberate contamination. In addition, he discussed the steps the Michigan Department of Agriculture has taken to put an emergency plan in place. Shari Rolando of APHL closed the meeting by thanking the participants and hosts and reminding the states that there would be a follow up on the action plans in six months.

Overall, the meeting was a valuable experience which allowed participants to candidly discuss concerns and successes regarding foodborne investigations. Indeed, one of the strengths of PulseNet is the ability to provide meetings on a regional level, allowing time for small group discussions. Because of this interaction, networking and planning, the overall goal of improving public health is achieved. 



### ANNOUNCEMENTS:

- Do you have a question or comment about CLIA regulations? Help is available!  
Contact information for CDC/Division of Laboratory Systems (DLS)/CLIA:  
**Website:** [www.phppo.cdc.gov/clia](http://www.phppo.cdc.gov/clia)  
**E-mail:** [phpdls@cdc.gov](mailto:phpdls@cdc.gov)  
**Phone:** (404) 718-1050
- The summaries of The 2006 PulseNet Update Meeting break out session two have now been posted to the APHL website at:  
[http://aphl.org/conferences/pulsenet\\_update\\_meeting\\_2006/Summaries\\_of\\_PulseNet\\_Breakout\\_Session\\_2006.pdf](http://aphl.org/conferences/pulsenet_update_meeting_2006/Summaries_of_PulseNet_Breakout_Session_2006.pdf)  
Many thanks to all of the note takers and moderators and to Steve Dietrich for compiling this summary.
- 2007 Annual Update Meeting will be in Providence, RI from April 16-19, 2007. Please visit <http://www.aphl.org> for details.

## Public Health Laboratory Data Integral to *E. coli* Outbreak Investigation

Sharon Rolando, Association of Public Health Laboratories, Washington, DC

Rapid analysis and reporting of laboratory results in Wisconsin, combined with the preliminary findings of an investigation in Oregon, helped federal agencies quickly detect and contain an outbreak of *E. coli* O157:H7. Active involvement laboratory and epidemiology staff in

both states was a hallmark of the early stages of this investigation. Collaboration with federal agencies, including the use of PulseNet data by CDC epidemiologists, helped to define the scope of what turned out to be a major nationwide outbreak associated with contaminated fresh spinach from California. Sound scientific practices used by New Mexico's Scientific Laboratory Division helped to further the investigation by pinpointing the product involved, followed by detection of contaminated product by public health laboratories in Colorado, Wisconsin, Nevada, Ohio, Pennsylvania, Utah, Illinois, and Arizona.

APHL organized three national conference calls to discuss laboratory issues related to the outbreak, with forty or more laboratories present on each call. Information was provided on sample handling, scientific protocols and reagents, the latest laboratory results, recommended testing algorithms from CDC, and the availability of free reagents through CDC's Director's Emergency Operations Center. APHL gratefully acknowledges the efforts of CDC staff that helped provide these needed reagents to our member laboratories.

The good work performed across the nation was recognized by CDC's Dr. Peter Gerner-Smidt when he told public health laboratories, "This outbreak is getting more and more interesting thanks to your scientific curiosity and enthusiastic work. It is a pleasure to work on a team like this." While many parts of this investigation went very well, future investigations can only improve by strengthening relationships among local, state, and federal food safety partners. Also, outreach to clinical laboratories may reduce the average time interval for clusters of infections to come to the attention of the public health community. 



## First and Second Year CDC EIS Officers

### First Year EIS Officers:



Casey Barton



Patricia Juliao



Christine Olson



Umid Sharapov



Anandi Sheth



Samir Sodha

### Second Year EIS Officers:



Wences Arvelo



Nicholas Gaffga



Sharon Greene



Manoj Menon



Ann Schmitz

## PUBLICATIONS:

1. Human Salmonellosis Associated with Animal-Derived Pet Treats --- United States and Canada, 2005. MMWR. June 30, 2006 / 55(25): 702-705.
2. Molecular Epidemiology and Cluster Analysis of Human Listeriosis Cases in Three US States. 2006. B Sauders, Y Schukken, L Kornstein, V Reddy, T Bannerman, E Salehi, N Dumas, BJ Anderson, J Massey, and M Wiedmann. J Food Protection 69 (7): 1680-1690.
3. *Vibrio parahaemolyticus* Infections Associated with Consumption of Raw Shellfish. MMWR. August 8, 2006 / 55(Dispatch): 1-2.
4. *Salmonella* Outbreaks in Restaurants in Minnesota, 1995 through 2003: Evaluation of the Role of Infected Foodworkers. Medus, C, Smith, K, Bender, J, Besser, J, and Hedberg, C. J Food Protection 2006; 69: 1870-1878.
5. Ongoing Multistate Outbreak of *Escherichia coli* serotype O157:H7 Infections Associated with Consumption of Fresh Spinach --- United States, September 2006. MMWR. September 29, 2006 / 55(38):1045-1046.
6. Importance of Culture Confirmation of Shiga Toxin-producing *Escherichia coli* Infection as Illustrated by Outbreaks of Gastroenteritis --- New York and North Carolina, 2005. MMWR. September 29, 2006 / 55(38):1042-1045.
7. Outbreaks of Multidrug-Resistant *Shigella sonnei* Gastroenteritis Associated with Day Care Centers --- Kansas, Kentucky, and Missouri, 2005. MMWR. October 6, 2006 / 55(39):1068-1071.

## POSTERS:

- Persistence of Distinct Clonal Groups in Most Commonly Found Serotypes of *Salmonella enterica* in Arkansas. R. N. Stefanova, K. Teng, R. J. Owens. To be presented at the 106th General ASM Meeting (May 21-25) in Orlando, FL.
- Use of Multiple-Locus Variably Number of Tandem Repeat Analysis (MLVA) to Further Characterize Shiga-Toxin-Producing *Escherichia coli* (STEC) O157 Clusters Detected by PFGE in PulseNet USA. Eija Hyytia-Trees, P. Gerner-Smidt, E. Ribot.
- A Geographically Dispersed Cluster of STEC O157:H7 Infections of Long Duration. An Outbreak or a Coincidence- A PulseNet USA Study. P. Gerner-Smidt, M. Joyner, E. Hyytia-Trees, C. O'Reilly.



ODH Staff: L-R Katherine Grandfield, Steve York, and Eric Brandt



Katherine Grandfield preparing plugs

## Lab Profile: Ohio

Tammy Bannerman, Ohio Department of Health Laboratory, Reynoldsburg, OH

The The Ohio Department of Health Laboratory (ODHL) is a part of the Division of Prevention for the Ohio Department of Health. The ODHL provides testing, consultation, and education to support public health programs throughout the state of Ohio and the nation. Established in 1898 as a Chemical and Bacteriological Laboratory by the Ohio State Board of Health, the ODHL was the fourth state laboratory in the nation. The ODHL is divided into three testing sections- microbiology, newborn screening and radiochemistry. The microbiology section is further separated into the following subsections: general microbiology, special microbiology and virology. Under the general microbiology subsection, the Molecular Unit is responsible for PulseNet activities.

In June 2006, the ODHL moved from its location on the campus of The Ohio State University in Columbus to the Ohio Department of Agriculture (ODA) campus in Reynoldsburg. This move allowed for the co-location of many lab-based state agencies, including ODHL, Ohio Environmental Protection Agency, and the Consumer Analytical Laboratory and Animal Disease Diagnostics Laboratory for the ODA.

The Molecular Unit began its participation in PulseNet in 1997. The current staff members in the Molecular Unit responsible for PulseNet activities

are Steve York (Coordinator for the Unit), Eric Brandt (primary staff member) and Katherine Grandfield (part-time staff member). These three members of the team are responsible for the routine pulsed-field gel electrophoresis (PFGE) analysis of foodborne isolates that get submitted to ODHL. In 2005 ODHL analyzed a total of 1496 foodborne isolates, including 8 *Campylobacter*, 155 *E. coli*, 26 *Listeria*, 1271 *Salmonella*, and 36 *Shigella* isolates. In addition to foodborne isolates, 334 nosocomial and other community-acquired isolates were analyzed. These included 46 *Acinetobacter*, 34 *Enterococcus*, 37 *Neisseria meningitidis*, and 179 *Staphylococcus* isolates.

In addition to providing PFGE support for epidemiologists in the ODH Bureau of Infectious Disease Control, local health departments, and infection

control personnel in hospitals, the Molecular Unit is involved in PFGE research efforts inside and outside of the laboratory. The ODHL has collaborated on several external projects to provide PFGE analysis for various organisms, including *Listeria*, *Staphylococcus* and *E. coli*. The Molecular Unit provides PFGE support for the ODA when foodborne organisms are isolated from various food products and environmental monitors. Finally, the Molecular Unit is responsible for analyzing bulk stools for Norovirus using polymerase chain reaction (PCR), PCR assays for virulent *E. coli*, and assisting with projects involving agents of bioterrorism.

The Molecular Unit has several goals to achieve over the next year. One of these goals is to continue the efforts to reduce the PFGE analysis turn-around time for both *E. coli* and *Listeria* isolates. The addition of the new CHEF Mapper and the incorporation of new staff members should help to make this occur. In addition, the Molecular Unit will begin work on the validation of PFGE for *Clostridium difficile* isolates and the use of PCR for further characterization of *E. coli* isolates for the *eae* and *E-hly* pathogenic markers.

The ODHL is proud to have been a part of the PulseNet since its inception ten years ago and we look forward to many years of continued collaboration with such an exciting group.



Ohio Department of Health, Columbus, OH

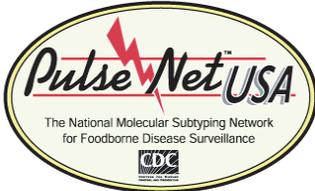
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Gerner-Smidt, Kara Cooper, Desmond Jennings,  
and Chris Perry.



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## INSIDE



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### Welcomes:

- **Ahmed ElSedawy** has joined the PulseNet team at CDC to work as coordinator of PulseNet International. As PulseNet International continues to grow, Ahmed will work with existing and future international projects and activities to ensure the continued success of the network.
- **Chris Perry** recently joined the PulseNet database team at CDC. He received his Bachelor of Science degree from the University of Georgia in May 2006. Chris will be dedicating his time to the National *Salmonella* Database, PulseNet News, and the 2007 update meeting agenda committee.
- **Debra Springer** has joined NC State Laboratory of Public Health's Microbiology group working in the PFGE lab. Debra is a Medical Laboratory Technologist II and also

an employee of the BT group. She recently passed PulseNet certification for both gel preparation and analysis of *E. coli* and *Salmonella*.

- **Erin Mulhern** is a new member to join the PFGE lab of Arizona State Public Health Laboratory. She is a Public Health Scientist II for PFGE, and she has moved from the Newborn Screening Section.
- The Hawaii Department of Health is proud to announce the addition of **Pamela O'Brien** to the PFGE lab. Pam has a BS in Biology from the University of Hawaii. She is currently being trained to perform all the PulseNet duties as she will eventually take over the PFGE program in Hawaii. In other news, **Precilia Calimlim** has been promoted to Microbiologist IV for the Bioterrorism Response Lab. Precy will continue with PulseNet along with Pam but will focus more on the FERN program.

- Mississippi Public Health Laboratory would like to welcome two new staff: **Daphne Ware** and **James Quinn**. Daphne Ware is the Technical Consultant for the Molecular Diagnostics Division and will be the contact person for the division. James Quinn is the tech who has been training to do PFGE. He's already working on certification for *Salmonella* and *E. coli* O157:H7.

- **Steven Stroika** has joined the PulseNet Central team at CDC. He received his Bachelor of Science degree from the University of Georgia in May 2004. Steven will be dedicating his time to the *Listeria* and *Campylobacter* databases.

### Farewells:

- **Kristy Kubota** has moved to California and has accepted a new position with UCLA Center for Public Health and Disasters as a Program Manager. Kristy has been an invaluable asset to the PulseNet team and will be sorely missed. Kristy has been a delight to work with and we wish her the best of luck with her career.

- **Natasha Pugsley**, Microbiologist III recently left the Vermont Department of Health Laboratory after eight years of service. Natasha played an instrumental role in the development of PulseNet activities in Vermont, and she will truly be missed both professionally and personally.

- The Massachusetts Department of Public Health said goodbye to **Dr. John Fontana** in November. John accepted a position as the Director of Biological Science with the Connecticut Department of Public Health. Over the years John has been the supervisor of the Virology, Rabies and HIV lab. Most recently, as a Director of the Bacterial Surveillance Laboratories, John headed the PFGE program, and the Enteric and Food laboratories. He will be missed in Massachusetts, but we wish him the best of luck in Connecticut.

- **Stephanie Kreis** accepted a position as the Tuberculosis Section Manager at the Arizona State Public Health Laboratory, and will no longer be doing PFGE on a routine basis. Her time will be dedicated to her new duties and responsibilities, but she will serve as a back up for Erin (see welcomes) on an as-needed basis. We wish Stephanie the best of luck with her future endeavors.

### HOW WOULD YOU LIKE TO RECEIVE THE PULSENET NEWSLETTER ?

Currently, subscribers to the PulseNet quarterly newsletter receive a hard copy in the mail. The newsletter is also available electronically on the WebBoard and on the PulseNet website ([www.cdc.gov/pulsenet/news.htm](http://www.cdc.gov/pulsenet/news.htm)). If you would like to stop receiving the hard copy version and either receive the electronic version via e mail or access it via the website or WebBoard, please send your request to the PFGE inbox at [pfge@cdc.gov](mailto:pfge@cdc.gov) with the subject line: PulseNet Newsletter.

