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The National Molecular Subtyping Network
for Foodborne Disease Surveillance



PulseNet News

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



VOLUME 5 • ISSUE 1 • 2005



really need! You don't need a lot of time to experience the Seattle area but you'll want to stay longer! Check out

<http://seattlepi.nwsourc.com/getaways/> for more ideas.

Hope to see you and your family in Seattle for a wonderful PulseNet Update meeting at the Westin Hotel in downtown. **CDC**

WELCOME TO SEATTLE, WASHINGTON

Ravi Pallipamu, Ramesh Gautam, Lito Badiable, Dr. David Boyle, Dr. Jinxin Hu, and Washington State Department of Health, Seattle, WA

Bring your outdoor gear when you come to the 9th Annual 2005 PulseNet meeting in Seattle. Come enjoy all the Northwest has to offer.

Lake Washington is a ten minute drive from downtown and the Cascade and Olympic Mountains are one hour away. Short ferry boat rides to various coastal towns leave several times a day. Take a day trip to Mount Rainer or Mount St. Helens. Or how about a weekend trip to Victoria, British Columbia, Canada on the Victoria Clipper though the San Juan Islands? Visit this website-<http://www.victoriaclipper.com/> for hotel



package information.

You don't have to like baseball to enjoy the fabulous view and games at Safeco Field Stadium. Nose-bleed ("view reserved") seats are cheap and still excellent seats. With the retractable roof, it makes it a no-brainer to attend. Visit <http://seattle.mariners.mlb.com> for ticket/schedule info. Of course there is the Public/Pike Place Market, a great place to spend hours shopping for things you don't

SOUTHEAST REGIONAL PULSENET CONFERENCE SUMMARY

Paola Bordon, PulseNet Database Administration Team, Centers for Disease Control and Prevention, Atlanta, GA

On February 9-10, 2005, over 50 PulseNet participants attended the first Southeast Area PulseNet Conference at the Crowne Plaza Ravinia hotel in Atlanta, Georgia. Attendees included representatives from all nine Southeast Area PulseNet labs:

(Continued on page 2)

PulseNet Conference (Continued from page 1)

Alabama, Florida (Jacksonville and Tampa), Georgia, Hawaii, Mississippi, Puerto Rico, South Carolina, and Tennessee. The United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) laboratories also sent representatives. This conference was modeled after past successful area meetings held in Minneapolis, MN in October

their Breakout Session groups. During the first Breakout Session, participants discussed their laboratory's successes and obstacles that affect foodborne illness and investigation and PulseNet functioning in their states. At the second Breakout Session, participants considered these obstacles and brainstormed solutions that had worked in other states and could be implemented. As day one ended, attendees agreed that strong communication lines had been

given by Dave Boxrud (MN) and John Besser (MN) about PFGE interpretation issues and the usefulness of PFGE for cluster identification. To open Breakout Session three, Lee Wotherspoon from Massachusetts State Laboratory Institute gave a brief presentation of successes following the Northeast PulseNet Regional Meeting. During Breakout Session three, representatives from each PHL created action plans to improve their food-



Southeast Regional Conference Participants, Atlanta, GA

2003 and Boston, MA in June 2004. The meeting objectives were to improve foodborne disease surveillance and response by uncovering state and regional PulseNet issues, to strategically address these issues, and to increase communication among and within public health laboratories (PHL). The conference opened with a PulseNet overview given by Kelley Hise (CDC), followed by presentations on epidemiology by John Besser (Minnesota Public Health Laboratory) and Craig Hedberg (University of Minnesota School of Public Health). Attendees from each state then shared summaries that highlighted their PulseNet-related obstacles, successes, organizational networks, and communicational mechanisms. These summaries offered opportunities for successful systems to be used as models for laboratories with PulseNet surveillance difficulties. After the state summaries, Jim Jones of the USDA-FSIS described the USDA's infrastructure and their role in PulseNet.

After the morning's state presentations and lectures, individual PHL representatives joined

established and they voiced confidence in their PulseNet strategies. In the evening, attendees convened at Brio Tuscan Grille to enjoy a delicious meal provided by APHL.

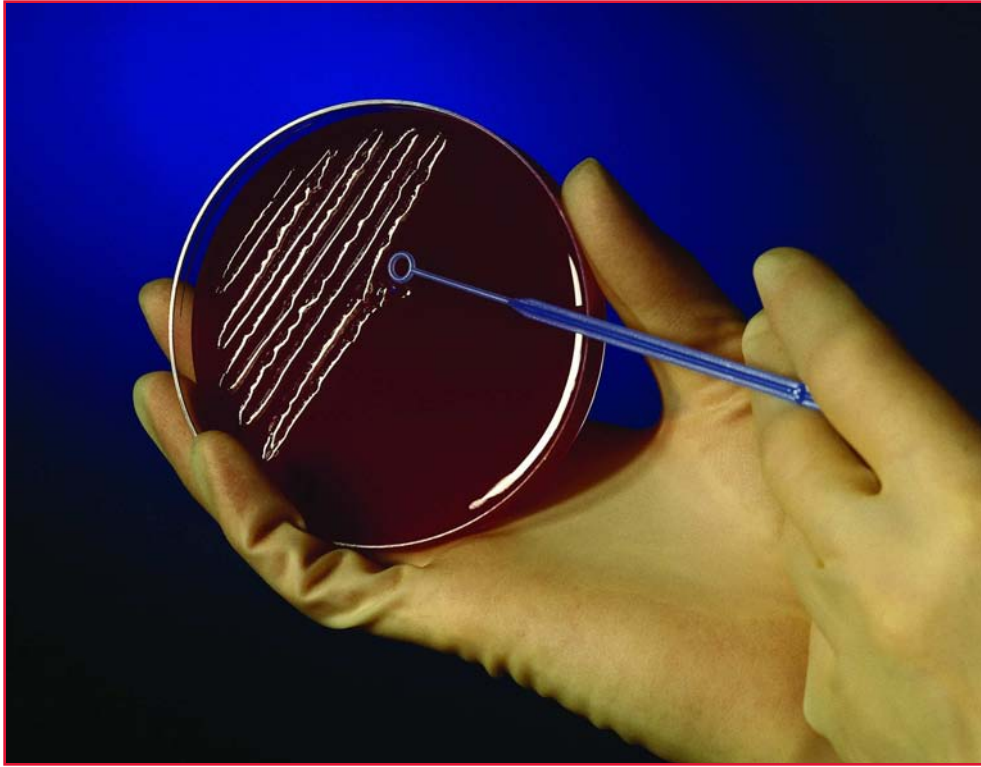
Day two began with a summary of the two breakout sessions, which highlighted commonly mentioned issues. The most common strengths or opportunities that were useful in addressing foodborne surveillance issues were dedicated staff, up-to-date PFGE equipment, strong communication between laboratory and epidemiology staffs, adequate funding, and student volunteers. Some of the weaknesses or obstacles included a lack of communication between laboratory and epidemiology staffs, isolate submission delays, lack of dedicated or trained staff, high turnover rates, inadequate data management systems, and insufficient funding. Ironically, areas found to be strengths in some PHL were highlighted as weaknesses in others, indicating the utility of area conferences in addressing common foodborne surveillance issues to improve regional surveillance.

After the summaries, presentations were

borne disease surveillance system by addressing issues discussed in previous breakout sessions. Attendees demonstrated a great deal of enthusiasm and team work and shared some good ideas for improving PulseNet function in each state.

Day two closed with presentations on Foodborne Disease Epidemiology at the CDC by Dr. Chris Braden, PulseNet International by Dr. Peter Gerner-Smidt, and Next Generation Subtyping Methods by Dr. Bala Swaminathan. These presentations were followed by an enthusiastic question and answer panel between the participants.

The Southeast Area PulseNet Conference was a successful first step to establish open communication within the region and to motivate participants to develop state action plans. The conference objectives were met and area conference calls will be held to maintain active participation and to continue state action plan development. Attendees proposed that a second meeting be held in one year. **CDC**



CERTIFICATION AND PROFICIENCY TESTING UPDATE

Christine Steward, PulseNet QA/QC Program, Association of Public Health Laboratories

The purpose of the PulseNet quality assurance/quality control (QA/QC) program is to allow accurate comparisons within the PulseNet national databases by ensuring that TIFFs of all gel images are comparable and of excellent quality with correct normalization and consistent band assignment. The QA/QC Program provides certification and proficiency testing of personnel at participating laboratories who submit TIFFs and/or analyses to PulseNet.

Certification.

Currently, certification is a one-time event per individual per PulseNet pathogen. Individuals can be certified for "gel image only" (i.e., laboratory methods for PFGE and image acquisition), "analysis only" (i.e., BioNumerics analysis

As of December 2004, 44 state public health, seven local (county or city) public health, eight Food and Drug Administration, and three United States Department of Agriculture laboratories have at least one person certified in analysis and/or gels for at least one PulseNet pathogen.

of gels and access to the national database), or both "gels and analysis" (i.e., PFGE, image acquisition, BioNumerics analysis, and access to the national database). To become certified, laboratorians should send an email to PFGE@cdc.gov requesting the strains that make up a certification set or sets. Since 2003, certification sets with only four strains each, with three strains restricted with two enzymes, have been available for *E. coli* O157:H7, *Salmonella*, and *Listeria*. *Shigella* and *Campylobacter* certification sets with seven strains each are also available. Each set fits on one 10-well gel. Once certification

gels are produced, documented, and analyzed by an individual, the TIFF and/or bundle certification files are sent from the participating laboratory to PFGE@cdc.gov where they are evaluated and a report is returned to the submitter. If analysis is certified, the individual will receive a SecurID device and login for on-line access to that database. The SecurID device is assigned to an individual, rather than a laboratory, and must be used only by that assigned individual.

As of December 2004, 44 state public health, seven local (county or city) public health, eight Food and Drug Administration, and three United States Department of Agriculture laboratories have at least one person certified in analysis and/or gels for at least one PulseNet pathogen. The number of U.S. PulseNet participating laboratories with individuals who are certified in analysis (i.e., able to access the national database) for *E. coli* O157:H7 (47 laboratories), *Salmonella* (52 laboratories), *Shigella* (29 laboratories), and *Listeria* (24 laboratories) is growing. However, these numbers indicate that not all U.S. participating laboratories are certified—and they should be. Compliance with certification procedures is required of all PulseNet participating laboratories. All laboratorians working in PulseNet-related activities should become certified. Laboratories with analysis-certified individuals can query and upload their own data to the national databases. This is an attainable goal for all participating laboratories.

In 2004, 167 **individual** certifications were completed. The current number of **individuals** at U.S. laboratories certified in analysis and/or gels for *E. coli* O157:H7 (100), *Salmonella* (128), *Shigella* (49), and *Listeria* (45) is impressive, but it can be increased. If you have submitted certification files and have not heard back from the Centers for Disease Control and Prevention (CDC) regarding certification status, please send an email to PFGE@cdc.gov, with the word "Certification" in the subject line of the email.

Countries other than the U.S. with certified PulseNet participating laboratories include Argentina, Canada, Denmark, Hong Kong, and New Zealand. These laboratories cannot directly access the U.S. national databases at CDC. However, they do send TIFFs and/or analyses to the CDC database team for uploading to the national databases.

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Testing Update (Continued from page 3)

Proficiency Testing.

Certification is valid as long as the laboratory successfully completes annual proficiency testing in which one strain for each PulseNet organism is sent to laboratories with individuals certified in analysis. Participants produce an in-house gel, analyze it, and upload it to the proficiency test database. In addition, all laboratories submit the analysis of a TIFF sent by CDC for each organism in which they are certified. Since all participants are analyzing the same TIFF, the "TIFF sent by CDC" component controls for gel-to-gel variation that could affect band finding from lab-

oratory to laboratory. In the Fall 2004 survey, band finding was graded according to the PulseNet Gel Analysis Guidelines, and TIFFs were graded according to the TIFF quality grading guidelines (visit the WebBoard for actual documents).

In the Fall of 2004, 56 laboratories (including one laboratory in Canada and one in Hong Kong) participated in proficiency testing. Eight laboratories submitted proficiency testing results for one pathogen, 27 laboratories submitted results for two pathogens, and 21 submitted results for three pathogens. Results for the Fall 2004 round were mailed from CDC to participating laboratories on January 11, 2005.

If your PulseNet participating laboratory does not have certified individuals or if you have questions or comments about certification or proficiency testing, contact PFGE@cdc.gov.

In any email correspondence, please include the words "Certification" or "Proficiency Testing" in the subject line. Our goal is to have all participating laboratories on-line. This will make comparisons with the national database more efficient and timely for all laboratories and will further speed the identification of outbreaks.

This will be my last update report because I am resigning my APHL contractor position effective December 2004. I have enjoyed working with such a dedicated group of people at CDC and at all the participating laboratories. The quality of the gels and of the gel analysis has improved tremendously over the two years I have worked on PulseNet QA/QC. I expect PulseNet to continue to improve and to grow dramatically. Take care and best wishes! **CDC**

PUBLICATIONS AND ABSTRACTS

- Hunter S, Vauterin P, Lambert-Fair MA, Van Duyn MS, Kubota K, Graves L, Wrigley D, Barrett T, Ribot E. **Establishment of a Universal Size Standard Strain for Use with the PulseNet Standardized Pulsed-Field Gel Electrophoresis protocols: Converting the National Databases to the New Size Standard.** *Journal of Clinical Microbiology.* 2005. 43(3): 1045-1050.
- Kornstein L. **Salmonella enterica Serotype Uganda Infection in New York City and Chicago.** *Emerging Infectious Diseases.* 2004. 10(9): 1665-1667.
- MacDonald PDM, Whitwam RE, Boggs JD, MacCormack JN, Anderson KL, Reardon JW, Saah JR, Graves LM, Hunter SB, Sobel J. **Outbreak of Listeriosis among Mexican Immigrants as a Result of Consumption of Illicitly Produced Mexican-Style Cheese.** *Clinical Infectious Diseases.* 2005. 40: 677-682.
- Mazurek J, Salehi E, Propes D, Holt J, Bannerman T, Nicholson LM, Bundesen M, Duffy R, Moolenaar RL. **A Multistate Outbreak of Salmonella enterica Serotype Typhimurium Infection Linked to Raw Milk Consumption—Ohio, 2003.** *Journal of Food Protection.* 2004. 67(10): 2165-2170.
- Olsen SJ, et al. **Multistate Outbreak of Listeria monocytogenes Infection Linked to Delicatessen Turkey Meat.** *Clinical Infectious Diseases.* 2005. 40: 962-967.

PULSENET WEBSITE GETS FACELIFT

Molly Joyner, PulseNet Database Administration Team, Centers for Disease Control and Prevention, Atlanta, GA



The PulseNet website is currently undergoing reconstruction to update and to improve its content and appearance. It is anticipated that the redesigned

PulseNet website will be online by summer of 2005. New additions to the website will include standardized PFGE protocols for pathogens tracked by PulseNet,

an updated list of PulseNet participants, and a more comprehensive description of PulseNet's role in public health. The updated material have an improved color scheme, updated logos, and pictures to give the PulseNet website a fresh new look. The redesigned PulseNet website will be updated regularly with staff additions, information regarding upcoming conferences and meetings, and the latest publications and pre-

sentations containing PulseNet data. Look for the new and improved PulseNet website in 2005! **CDC**

HOW WOULD YOU LIKE TO RECEIVE THE PULSENET NEWSLETTER?

Currently, all subscribers to the PulseNet 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). 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 the WebBoard, please send your request to the PFGE inbox at pfge@cdc.gov with the subject line: PulseNet Newsletter.

TEXAS DEPARTMENT OF STATE HEALTH SERVICES

Ana Maria Valle-Rivera, PhD, Molecular Biology Team Leader, Laboratory Operations Unit, Texas Department of State Health Services, Austin, Texas

The Laboratory Operations Unit (LOU) of the Texas Department of State Health Services, formerly the Texas Department of Health, serves as the state reference laboratory and support for epidemiologists investigating infectious disease outbreaks. The Molecular Biology (MB) Team within the LOU is responsible for molecular diagnostics and PulseNet activities.

When the MB Team joined PulseNet as an area lab in 1996, the original focus was pulsed-field gel electrophoresis (PFGE) of foodborne pathogens and detection of *E. coli* O157:H7 by PCR. In 2003, the Microbiological Investigations Team merged with the MB Team which had been responsible for molecular diagnostics. The newly enhanced MB Team is responsible for strain typing of all *Salmonella* sp. (147 serotypes), *Shigella* sp., *E. coli* O157:H7, and *Listeria monocytogenes*. PFGE is also used to type *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Streptococcus pneumoniae*, *Serratia marcescens*, *Burkholderia cepacia*, *Vibrio vulnificus*, Group A *Streptococcus* (GAS), *Streptococcus pyogenes*,

Pseudomonas aeruginosa, *Acinetobacter* sp., and a few rare nosocomial agents that are submitted to the lab. Also, the MB Team performs all *Salmonella* sp. and *Shigella* sp. serotyping. The use of PFGE as a start-up point for the serotyping has helped streamline the process and saved thousands of dollars in antisera.

The molecular diagnostic activities include *Bordetella pertussis* real-time (RT) PCR, norovirus RT-PCR and confirmation of *E. coli* O157:H7 by PCR. The MB Team assists with arbovirus surveillance by testing horse brain tissues and mosquito pools for West Nile Virus and St. Louis encephalitis virus by real-time RT-PCR. Specimens for California viruses are tested as necessary. The LOU is currently involved in a study of Hepatitis G prevalence by RT-PCR. The samples in the study are a group of Hepatitis C positive samples previously genotyped in the lab by RT-PCR. This study is being performed with the assistance of an undergraduate student from the University of Texas at Austin as part of a new internship program. Additional activities include assisting the Bioterrorism Investigation Team as needed.

Since 2003, the LOU has



BACK ROW: Eric Casey, David Stringer

FRONT ROW: Chun Wang, Ana Maria Valle-Rivera, Eileen Huston Prentice, Adrienne Garcia, Grace Kubin

made great achievements in meeting the goals as a PulseNet area lab. Before 2003, it was understaffed and had chronic employee turnover, causing certification issues. In less than a year, the lab has caught up in all aspects and continues to improve. The LOU has increased the number of organisms that are typed and uploaded to the national database by including *Shigella* sp. Previously, the LOU only performed PFGE for *Shigella* outbreaks as requested.

Since January 2004, all *Listeria* and *E. coli* O157:H7 have been serotyped and uploaded to the national databases; over 870 *Salmonella* sp. were serotyped, subtyped, and uploaded; and 386 *Shigella* sp. (of which 101 were uploaded) were serotyped.

There are seven full-time microbiologists in the lab: Grace Kubin, Ph.D. (PulseNet

activities: CDC certified in *Shigella* sp.), Eric Casey (PulseNet activities: CDC certified for *Salmonella*, *Shigella*, *E. coli*, *Listeria*), David Stringer (PulseNet and serotyping activities: CDC certified for *E. coli* and *Listeria*), Eileen Huston Prentice, M.S. (PulseNet and serotyping activities: CDC certified for *Shigella*), Adrienne Garcia (molecular diagnostic activities), Chun Wang, M.S. (molecular diagnostic activities) and Ana Maria Valle-Rivera, Ph.D. (Team Leader). The LOU currently houses two EID Fellows: Stephanie Volz, M.S. and Ellen Trimarco (both are involved in molecular diagnostic projects) and one fall intern from the University of Texas. The MB Team is highly motivated to add new protocols and techniques to meet the demands of future investigations for foodborne and non-foodborne outbreaks. **CDC**

CALL FOR CONTRIBUTIONS

The editors welcome any contributions for the "PulseNet News" newsletter in the form of short articles, recent publications, conference abstracts, news, and anything else related to PulseNet. Please direct all submissions to the APHL PulseNet Program Manager, Sharon Rolando (srolando@aphl.org).

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Welcomes

- **Crystal Johnston** recently joined the Epidemiologic Support Group at the Virginia Division of Consolidated Laboratory Services. She will be working in the molecular typing laboratory providing testing support for foodborne and other infectious disease surveillance initiatives.
- **Ali Fernandez** and **Kimmi Schrader**, both public health microbiologists, recently joined the California Department of Health's PFGE section in the Enterics Unit.
- **Brian Bernier** has been trained by the Maine PFGE laboratory to provide back up and surge capacity for the PFGE Lab during the busy season.
- **Vincent Cummings** joined the PFGE team at the Pennsylvania Public Health Laboratory. He comes to us from the laboratory's Training and Proficiency Testing Section. His extensive knowledge of reptiles (his hobby) and background in math and science teaching will be an asset to the PFGE section.
- **Jennifer Everman**, Microbiologist III, has recently transferred into the Kentucky

PFGE lab from the TB lab. Jennifer will be responsible for all lab duties related to PulseNet and is our NARMS contact.

- **Robin Cotten** has recently transferred from the Kentucky BT lab to a position which will serve more of an epi role for the PFGE lab. Robin will help us improve communication between our epi department and the PulseNet lab.
- **Karim George** is now the new Virology Supervisor at the Kentucky Department of Health. Kentucky PulseNet activities will transfer from the Bacteriology Section to Virology.
- **Mark Tyndall** has joined the Massachusetts PFGE laboratory as a Bacteriologist. Previously, Mark worked for John Fontana in the Massachusetts Antibiotic Resistance Surveillance lab.
- **Kaye Eckmann** and **Lito Badiable** will be performing PulseNet work for the Washington State Public Health Laboratory.
- **Key Griego** has been promoted to work with Debbie Sena-Johnson in the New Mexico PFGE lab and also to work on grant applications.
- **Amy Pierce** is the new PFGE supervisor

at the Missouri State Public Health Laboratory.

- **Mike Sharp**, a new Microbiologist II in the Tennessee PFGE lab, is currently being trained on the PFGE bench and has completed BioNumerics training.
- **Lixia Liu**, the supervisor of the Virology/Immunology labs, is the new technical PulseNet supervisor at the Indiana State Department of Health.
- **Veronica Erwin**, a Microbiologist in the Food/Dairy Lab, will be performing PFGE and analysis at the Indiana State Department of Health.
- **Keith Obye**, a Microbiologist in the Hepatitis/Immunology Lab, will be performing PFGE and analysis at the Indiana State Department of Health.

Farewells:

- **Ravi Pallipamu** first joined the microbiology division of WA PHL 11 years ago and was initially involved with *Chlamydia* and then TB. In 1999, Ravi began working in the PFGE facility and has been the lead since then. Ravi's great level of commitment to the PulseNet program led to a PulseStar Award in

2004. Sadly, for PulseNet and the WA PHL, Ravi's family commitments have led him to further his career in Norway. We will miss his skills and his irreplaceable character and wish him every success in the future.

- **Kimberly Ferguson**, a member of the Epidemiologic Support Group at the Virginia Division of Consolidated Laboratory Services, left in February to pursue a career opportunity at Johns Hopkins. We wish her well in her new career endeavor.
- **David Elliott**, a member of the Epidemiologic Support Group at the Virginia Division of Consolidated Laboratory Services, has recently assumed a new position within our group. His duties will involve the developing and validating of sequencing based methods for identification and characterization of infectious agents.
- **Lea Kelso** has left the PFGE section of the California Department of Health to join the Public Health Microbiology training. We wish her the best of luck.
- **David Stringer** left the Texas PFGE lab to work for the Dallas County in BT activities. We wish him well.