Volume 1, Issue 2, Fall 2001

PulseNet News

The National Molecular Subtyping Network for Foodborne Disease Surveillance
State & Local Public Health Laboratories in the United States
PulseNet North - Canada

PulseNet News salutes our members across the nation who are responding to the bioterrorism events and investigations. Your extraordinary efforts are sincerely appreciated.

SECRETARY THOMPSON ENCOURAGES FOODBORNE BIOTERRORISM PREPAREDNESS.

Tommy Thompson, Secretary of Health and Human Services, has publicly stated and testified before the U.S., Congress that he is concerned about the security of the nation's food supply. The nations food supply presents an attractive target for potential bioterrorists because food production, processing, and distribution systems are highly centralized.*[1] Intentional contamination of foods with disease-causing microorganisms or their toxins could sicken large numbers of people, leading to widespread panic in the population. A covert bioterrorist attack on the United States food supply would first manifest itself as an increase in foodborne illness cases. If such an attack should occur, local and state public health laboratories will play a critical role in recognizing related clusters of illnesses by rapidly typing and subtyping bacteria isolated from patients. The PulseNet network will facilitate rapid detection of clusters by enabling real-time comparison of bacterial DNA fingerprints at the national level. Now, more than ever, real-time subtyping of foodborne disease-causing bacteria is essential for early identification of unintentional and intentional food contamination events. Early recognition of foodborne disease clusters and epidemiologic investigation of these clusters will help prevent more people from becoming ill and may save lives.

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WELCOME TO THE NEW PULSENET PROGRAM MANAGER AT APHL

Sharon Rolando has assumed the new position of PulseNet Program Manager for the Association of Public Health Laboratories (APHL). Shari began working at the APHL office in Washington, DC on September 26, 2001. She is a certified Medical Technologist (ASCP) and earned her Master of Health Science degree from the Johns Hopkins School of Public Health in 1998. She worked in clinical microbiology laboratories for 9 years and has experience in molecular diagnostics. She spent this past year as an APHL/CDC Emerging Infectious Diseases training fellow at the State Laboratory Institute in Boston, Massahusetts, where she worked closely with their PFGE staff.

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PULSENET DATABASES ONLINE

By Susan Hunter, Microbiologist / Chief, PulseNet National Database Administration Team, CDC

The *E. coli* online database is being actively used by many PulseNet participants. Currently, 27 participating laboratories have received SecurID fobs to enable them to authenticate through the CDC firewall and access the PulseNet online server. To receive a SecurID fob, an individual must successfully complete the analysis portion of the *E. coli* certification. Gel images submitted to CDC must be analyzed by personnel who have successfully completed the TIFF image portion, which shows that plugs can be prepared and gels can be run according to the standardized protocols. We are currently able to issue a maximum of two fobs to each participating laboratory. Of the 27 laboratories that have received fobs, two have not yet been able to successfully connect because of local firewall or computer problems. During the month of October 2001, patterns from 455 isolates were submitted to the server either by direct submission (258) or by e-mailing the profiles to CDC for analysis and subsequent submission by the PulseNet Database Administration team (197).

Persons who are submitting to the online server are reminded to always use the data entry screen and pulldown menus for entering text data. Then, use the submit button to send both your text data and patterns. Questions related to the online system or CDC PulseNet BioNumerics can be sent to PFGE@CDC.GOV, Kristy Kubota or Wade Ivy 404-639-4558, or Susan Hunter 404-639-1749.

The database administration team is working to establish both *Listeria* and *Salmonella* online databases in the near future. We expect to have scripts for client setup for these databases early 2002.

The editor welcomes any contribution for the "PulseNet News" Newsletter in the form of short articles, news of recent publications, conference abstracts, news and anything else related to PulseNet. Please direct all submissions to the editor (ztu1@cdc.gov)

Call For Contributions.

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Publication of: *The Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Bacterial and Mycotic Diseases, Foodborne and Diarrheal Diseases Branch.*

ISSUE CONTENT

PUBLICATIONS & ABSTRACTS FOR FALL 2001

The molecular epidemiology and antimicrobial susceptibility of outbreak and sporadic *Salmonella enterica* serotype Typhi isolates in Florida. Fiorella PD, Baker R, Katz D, Farah R, and Taylor J. 101st American Society of Microbiology General Meeting, Orlando, FL, 21 – 24 May 2001.

Multistate outbreak of *Listeria monocytogenes* infections linked to deli turkey meat. Olsen SJ, Evans M, Hunter S, et al., 38th Annual Meeting of the Infectious Diseases Society of America, San Francisco, CA, 25 – 28 October, 2001.

Multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. Breuer T, Benkel DH, Shapiro RL, et al., Emerging Infectious Diseases Journal, Vol. 7, No. 6 Nov–Dec 2001.

PERSONAL ACHIEVEMENTS

Michele Bird, Guest Researcher/Microbiologist in the PulseNet Methods Development and Validation Laboratory at CDC, completed a marathon in Athens, Greece, on November 4, 2001 to raise money for a young boy in the Atlanta, Georgia, area who suffers from arthritis. Michele completed the 26.3-mile marathon with the same dedication and determination that she began her training with a year ago. She set a goal to raise $4,000, which she met and exceeded with the help and support of many friends, family and coworkers. She raised the money in numerous ways – most by "Dear Friend" letters to people that she knew, where she shared Jesse's story, explained the mission of Joints in Motion and asked for donations and prayers for the National Arthritis Foundation.

PULSENET GOES TRANSATLANTIC

A transatlantic conference call signaled major progress in the development of PulseNet Europe. The Centers for Disease Control and Prevention (CDC) and the United Kingdom Public Health Laboratory Service (PHLS) agreed on standard PFGE protocols and determined how to best proceed with the collaboration. It was agreed that BioNumerics software should be used to facilitate the comparison of data generated both in the United States and Europe. This was particularly important because international participants are unable to submit directly to the CDC server. This also alleviates problems associated with ownership of the data stored by both parties. Database managers in the United States and the UK will coordinate the identification of clusters of isolates from potential outbreaks and the surveillance of foodborne pathogens on both sides of the Atlantic Ocean. Further discussions were scheduled to take place at the Enter-Net workshop in Athens, Greece in late September but the conference was postponed following the recent tragic events in the United States. CDC has invited representatives from PulseNet Europe to attend forthcoming BioNumerics workshops for PulseNet participants in the United States next year as well as the 6th Annual Update meeting scheduled to take place in Michigan during the spring of 2002.

CANADIAN LISTERIOSIS REFERENCE SERVICE ADDS RIBOTYPING

By Franco Pagotto, Bureau of Microbial Hazards, Health Products and Food Branch, Food Directorate, Health Canada

The National Laboratory for Enteric Pathogens and the Bureau of Microbial Hazards has created a listeriosis reference service (LRS) under the direction of Dr. Clifford Clark, Franco Pagotto, and Jeffrey Farber. A major focus of this service is providing a comprehensive database of all isolates (clinical, environmental and food) in Canada for use as a resource for outbreak investigations, surveillance, and other microbiological investigations. The LRS will house a common molecular typing database, using PFGE as the main typing method, to track cases of human foodborne listeriosis. Profiles will be established and stored for clinical, food, environmental, and possibly animal strains of *Listeria monocytogenes*. We are pleased to announce that the LRS has recently purchased the automated RiboPrinter® Microbial Characterization System (DuPont Qualicon), and will be including a ribotype profile for all isolates being analyzed. Other functions of the LRS include assisting the provincial epidemiologists and public health laboratories with a central reference laboratory that is capable of isolating *L. monocytogenes* from food, clinical and environmental samples associated with sporadic cases and outbreaks of foodborne listeriosis.

THE PULSENET PROFICIENCY TESTING PROGRAM BEGINS

By Susan Van Duyne, Microbiologist / PulseNet Database Administration Team, CDC

The PulseNet Proficiency Testing (PT) Program began the last week of October 2001. The objective of this program, as stated in the Quality Assurance/Quality Control (QA/QC) Manual for PulseNet Standardized Pulsed-Field Gel Electrophoresis, is " To provide an ongoing program of external testing to ensure that laboratories participating in PulseNet maintain a satisfactory level of performance for PFGE or other molecular subtyping methods." (Section I.) The PT program is designed to test, on an annual basis, the quality of gels being produced by laboratories participating in the PulseNet system. Laboratories who fail to maintain high-quality results for any organism will no longer be certified for that specific organism; their online access to that specific PulseNet National Database will be terminated until retraining and successful re-certification occurs.

This initial "challenge" consists of one isolate each of *E. coli* and a nontyphoidal *Salmonella*. Each isolate of *E. coli* and *Salmonella* should be restricted and run with both *Xba*I and *Avr*II (*Bln*I). Additional organisms will be added to the challenge in subsequent years. The PulseNet participating laboratories have been divided into two groups; one group received their PT challenge in November 2001, and the second group will receive their PT challenge in Spring 2002.

The participating laboratories are requested to use this as an internal QA event. Each laboratorian who does PFGE testing for PulseNet should run the unknown PT isolates. However, only ONE set of patterns (*Xba*I and *Avr*II [*Bln*I]) per organism per laboratory should be submitted as your "official" PT result. The PT unknowns are to be run on routine gels without special procedures.

If you have additional questions, please contact Susan Van Duyne, mdv9@cdc.gov, 404-639-0186

PULSENET AND PULSENET NORTH: A SUCCESSFUL PARTNERSHIP

By Susan Van Duyne, Microbiologist / PulseNet Database Administration Team, CDC

Since early 2000, when Health Canada formally joined the PulseNet network as PulseNet North, a number of cross-border outbreaks have been successfully investigated. This collaborative effort reached a high point early in 2001 with the investigation of an outbreak of *Salmonella* Enteritidis PT30 infections. This unique phage type had rarely been seen but was suddenly identified from several Canadian provinces starting in November, 2000.

Extensive investigation by Canadian public health authorities implicated raw almonds as the probable vehicle for the outbreak. The almonds were identified as having come from the United States (California). A request came from PulseNet North in April 2001 to look for this phage type and associated pulsed-field gel electrophoresis (PFGE) pattern(s) in the United States, but no PFGE patterns in the PulseNet National *Salmonella* Database appeared to match these new, unique patterns from the almond outbreak. However, after requesting all PulseNet participating laboratories to test a portion of the *S.* Enteritidis isolates they were receiving by PFGE, United States cases related to the outbreak were discovered.

Further investigations by the U.S. Food and Drug Administration and California Department of Agriculture began to recover isolates of the implicated *S.* Enteritidis from various locations in the chain of almond production. These matched the clinical isolates from the outbreak by phage type and by two enzymes with PFGE.

Close cooperation between the various PulseNet laboratories in the United States and Canada and between the epidemiologists in each public health agency successfully identified the vehicle of infection and helped control the outbreak. Guidelines to the almond industry are currently being developed to help reduce this type of contamination in the future.

QUESTIONS, ANSWERS AND TROUBLESHOOTING

PulseNet News will feature a questions, answers and troubleshooting column in each issue. The aims of the column will be the dissemination of knowledge and new developments within the PulseNet network.

Troubleshooting Tip: Curved Lanes On PFGE Gels

By Stephen Dietrich, Michigan Public Health Laboratory.

We recently had a problem with curved, distorted lanes on gels run on each of our four CHEF units. One problem that can cause this effect is reduced buffer flow rate resulting in insufficient or nonuniform buffer cooling. BioRad recommends a flow rate of 1 liter per minute, but on checking our CHEF units I found that the flow rates were much lower than this. One cause of decreased flow rate is clogging of the exit pores by pieces of agarose from the gel; this can occur even with the agarose trap installed in the gel chamber. To unclog the pores, we reversed the direction of buffer flow, forcing the agarose pieces from the exit pores. The flow rate was then returned to 1 liter per minute and the problem with curved lanes was resolved. We have since established a regular preventive maintenance procedure to check and adjust the flow rate. The troubleshooting guide in the manufacturer's instructions lists some additional causes of curved lanes, including dirty and corroded electrodes. If you wish to share your experience, you can either email the PulseNet listserv ecolilistserv@cdc.gov or Stephen E. Dietrich at DietrichS@state.mi.us.

Secretary Thompson Encourages Foodborne Bioterrorism Preparedness - Continued from page 1

*1 Maskanka SE, Zirnstein G, Sobel J, Swaminathan B. 2001, Foodborne Pathogen and Toxin Diagnostics: Current Methods and Needs Assessment from Surveillance, Outbreak Response, and Bioterrorism Preparedness Perspectives, p. 143 – 163, Layne SP, Beugelsdijk TJ, Patel CKN ed,, Fire Power in the Lab, Joseph Henry Press, 2101 Constitution Avenue, N.W. Washington, DC 20418.

Welcome to the New PulseNet Program Manager at APHL - Continued from page 1

The main responsibilities of the Program Manager will be to evaluate the activities of the PulseNet participating laboratories, to facilitate communication between participating laboratories, their area laboratories and CDC, and to coordinate the training needs of all PulseNet members.
Additionally, the Program Manager will help organize the PulseNet Update Meetings and schedule conference calls between laboratories, epidemiologists, and CDC, when necessary to meet the ongoing needs of the program.
Your comments and suggestions for improvements to the PulseNet Program can be forwarded to Shari by e-mail at srolando@aphl.org or by phone at 202-822-5227, ext 205. She looks forward to meeting many of you at the next PulseNet Update meeting in Michigan.

THIOUREA USEFUL FOR SUBTYPING "NONTYPEABLE" STRAINS BY PFGE

By Mary Ann Lambert-Fair,
Research Microbiologist, CDC

Although PFGE is a very useful tool for characterizing bacteria in epidemiologic studies, outbreaks and other situations, there are often strains, serotypes, or species of various bacteria that will not type with PFGE. When this happens, only a heavy "smear" of DNA is seen in the lower half of the gel (Fig. 1, Lanes 3, 4, 6-9). Often, the restriction is repeated or the culture regrown and new plugs made in case some mistake had been made in processing the culture. Eventually, one may decide that this is an "untypeable" strain unable to be typed by conventional PFGE. Although many attempts had been made to subtype these "untypeable" strains using PFGE, none were successful in our laboratory until two separate reports describing the use of thiourea in the running buffer were published in 2000 in the Journal of Clinical Microbiology.[*1] We repeated the work of these investigators using several Enterobacteriaceae of clinical interest that had previously been considered "untypeable" by PFGE, including strains of *E. coli* O157:H7 phage type 31, and three serotypes of *Salmonella* (Ohio, Makumira, and Saintpaul). The results of this work were reported at the 2001 PulseNet Update Meeting in Richmond, Virginia.

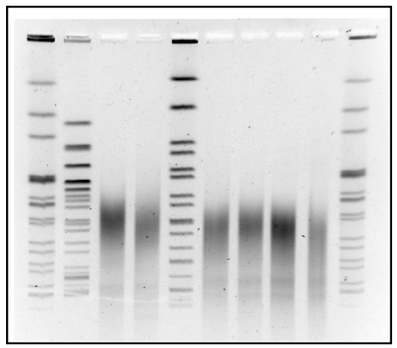

Fig. 1, Lanes 3, 4, 6-9

PFGE plugs of the "untypeable" strains were made and restricted as described in the PulseNet Standardized Protocol. Before the gel was placed into the chamber, 860 µl of a 10-mg/ml aqueous solution of thiourea (Thiocarbamide; $(NH_2)_2CS$) was added to 2.2 liters of the running buffer for a 50-µM concentration. After the electrophoresis, the gel was stained, destained and imaged as described in the standardized protocol. Addition of the thiourea prevented the degradation of DNA by protecting the DNA in isolates with labile modification sites by scavenging reactive radicals that are formed at the anode during electrophoresis (Fig. 2).

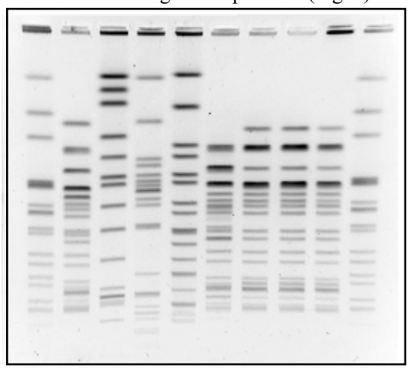

Fig. 2, Lanes 3, 4, 6-9

It did not affect the migration banding pattern of the standard strains or other isolates, which do not have this specific mutation in the DNA (Fig. 2., Lanes 1, 2, 5, 10).

Thiourea is an organic compound that resembles urea. Its commercial applications are limited and it is primarily used as a fixative in photography, in insecticide and in the textile industry. According to the Material Safety Data Sheet (MSDS), it can cause irritation to skin, eyes and respiratory tract; it may also cause allergic skin reactions and is a possible cancer hazard. Thus, there are some concerns about weighing, handling, and disposing of thiourea. The CDC Office of Health and Safety was contacted before we made and used the thiourea solution in the running buffer and they determined that the thiourea solution was not a chemical hazard in the dilution and quantities that we were using, and the spent buffer could be flushed down the sink with additional quantities of water. We use disposable gloves when handling the gel, the gloves are put in discard pans with other disposable items and autoclaved after use. The spent buffer is released directly into the sink with running water or drained into a large flask or other container and then poured down the sink with care to avoid any splashing, and flushed with additional water. Because the long-term effects of thiourea on the tubing and electrophoresis chamber are not known, two liters of reagent grade water are circulated for 15-20 minutes through the lines and electrophoresis chamber immediately after completing electrophoresis with thiourea. This rinse water is drained from the lines and chamber and flushed down the sink with additional water. The original thiourea solution that was made in late 2000 has been stored on the laboratory shelf at room temperature for at least 9 months with no apparent change in effectiveness. We have not noticed formation of a white coating on the electrodes as some reports have described.

The next edition of the PulseNet QA/QC Manual will contain a section describing the use and handling of this chemical for PFGE. In the interim, here are some guidelines to use in your laboratory:

* **Use personal protective equipment (eye protection, gloves and mask) when weighing thiourea.**
* **Weigh thiourea in a chemical fume hood, if one is available.**
* **Do not contaminate the balance and surrounding area with this chemical.**
* **Dispose of the weighing paper and wipe the balance and weighing area with a moistened towel to remove any spills.**
* **Use a disposable spatula, such as a wooden tongue depressor, to remove the chemical from the bottle.**
* **Recap bottle tightly after use.**
* **Discard all items that come in contact with thiourea as hazardous waste, according to the guidelines of your institution.**
* **Flush solutions of thiourea down the sink with copious amounts of water.**

If you have additional questions on the use of thiourea for subtyping "nontypeable" strains by PFGE, please contact Dr. Efrain Ribot, eyr4@cdc.gov, 404-639-2153 or Mary Ann Lambert-Fair mal3@cdc.gov, 404-639-3764.

[*1] Corkill JE, Graham R, Hart CA, Stubbs S. 2000, Pulsed-Field Gel Electrophoresis of Degradation-Sensitive DNAs from *Clostridium difficile* PCR Ribotype 1 Strains. J. Clin. Microbiol. **38**:2791-2792.

Römling U, Tümmler B. 2000, Achieving 100% Typeability of *Pseudomonas aeruginosa* by Pulsed-Field Gel Electrophoresis. J. Clin. Microbiol. **38**:464-465.

MARK YOUR CALENDARS!

PulseNet 6th Annual Update Meeting, 8-10 April 2002, in Michigan

The 6th Annual PulseNet Update Meeting will be hosted by the Michigan Public Health Laboratory Services and is scheduled to take place on 8th – 10th April 2002, at the Sheraton Inn in Ann Arbor. The proposed theme for the 2002, meeting will be **"The Role of PulseNet in the Public Health Response to Bioterrorism."** If any members have suggestions for session topics and/or speakers, please forward them to the Shari Rolando at srolando@aphl.org.

Each participating laboratory should plan to send at least one representative to the annual PulseNet Update Meeting. Hope to see you there!

PulseStar Awards

Please begin to consider possible candidates to be nominated for the PulseStar Awards that are presented annually by FDDLS/CDC and APHL.

The award was initiated in 1998 to recognize those individuals whose outstanding contributions to PulseNet during the previous year resulted in significant improvements to the procedures (either laboratory or computer) or communications related to PulseNet. As many as three awards will be presented annually to those PulseNet participants who have made outstanding and significant contributions to PulseNet activities in public health. The award consists of a plaque and a check for $500 provided by APHL. The nomination forms and criteria will be posted on the Listserv early in the new year.

CDC PulseNet Task Force

Farewells to the PulseNet Task Force.

Farewell to **Nicole Tucker**, who recently accepted an epidemiologist position with the Foodborne and Diarrheal Diseases Branch at CDC. Farewell also to **Heather Noll**, who is studying Veterinary Sciences at the University of Tennessee, **John Allan,** who is continuing his education at the University of Georgia where he is studying for a Master's degree in Food Microbiology, and **Ben Holland** who is studying at Mercer Medical School. We wish Nicole, Heather, John and Ben much success in their new endeavors.

Welcome to the new CDC PulseNet Task Force.

Frank W. Virgin Jr., came from the University of Georgia, where he was a Laboratory Assistant in the Department of Botany/Genetics. He was involved in research, using allozyme analysis to measure genetic drift and the population diversity of rare southeastern plant species. Frank's responsibility with the PulseNet Database Administration team will be cluster identification and surveillance of various serotypes of *Salmonella*. Frank is a graduate of the University of Vermont and will be continuing his education at the Medical College of Georgia in August 2002.

Wade Ivy, III, came from the Viral and Rickettsial Zoonoses Branch at CDC, where he investigated the association between rabies-infected raccoons and spillover into skunk populations. His responsibilities with the PulseNet Administration Database Team are cluster identification and surveillance of *Escherichia coli* O157. Wade is a graduate of Grambling State University in Grambling, Louisiana, and plans to continue his education by pursuing a PhD in epidemiology of infectious diseases.

Kimberly McGill, a graduate student at Georgia State University, is currently pursuing a masters degree in biology after completing her undergraduate studies at Clark Atlanta University, Atlanta Georgia. Her role on the PulseNet Team is manager for the *Shigella* database.

Rebecca Middendorf previously interned at the CDC in the Division of Viral and Rickettsial Diseases. Currently, she is working with the PulseNet Methods Development and Validation Laboratory assisting with a variety of projects. Rebecca is a graduate of Agnes Scott College in Decatur, Georgia and is planning on continuing her education with a Masters in Health Policy and Management.