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PulseNet News

The National Molecular Subtyping Network for Foodborne Disease Surveillance

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

Welcome to the first issue of PulseNet News

The PulseNet Task Force at CDC would like to welcome readers to the first issue of the PulseNet Newsletter. The aim of the Newsletter is to communicate new achievements and developments to the PulseNet Network.

Issue Content

The 5th Annual PulseNet Meeting Hailed as a Success

The fifth annual PulseNet update meeting was hosted by the Commonwealth of Virginia Division of Consolidated Laboratories Services (DCLS) at the Omni Hotel, Richmond Virginia 2-4 May, 2001. One hundred twenty-six delegates, representing 45 states, and the Food and Drug Administration and the U.S. Department of Agriculture, as well as international representatives from Canada (PulseNet North) and the United Kingdom Public Health Laboratory Service (PHLS) attended the meeting. Special thanks go to Denise Toney and Judy Carroll of DCLS for organizing the meeting.

The meeting opened with introductions and welcomes from Rosemary Humes, Director, Infectious Disease Programs, Association of Public Health Laboratories (APHL), James Pearson, Deputy Director, DCLS and Robert Stroube, Director of the Office of Epidemiology, DCLS. James Hughes, Director of CDC National Center for Infectious Diseases sent a “special audio-visual message” in which he described PulseNet as a shining example of a scheme that answers the four goals of the CDC Emerging Infectious Disease strategy. He also praised PulseNet for providing timely disease surveillance facilitating rapid public health response, or in his own words, "public health action in real-time."

The first scientific session was opened by Bala Swaminathan, Chief of the Foodborne and Diarrheal Diseases Laboratory Section at CDC, who described some of the achievements and progress made by PulseNet in the preceding 12 months and the goals and objectives for 2001. During the following scientific sessions a diverse range of topics were presented and discussed, including real-time subtyping, expansion and improvements to protocols, questions and answers, and transition the new software, BioNumerics. The "Real-Time Subtyping" session was chaired by John Besser from the Minnesota Public Health Laboratory, and included a panel discussion on practical approaches for real-time subtyping. New PFGE protocols were introduced for *Campylobacter jejuni*, *Clostridium perfringens*, *Vibrio parahaemolyticus* and *V. cholerae*, and the current status of the *Listeria monocytogenes* PulseNet project was reviewed. In another session called "Improvements to Protocols" a possible ‘universal size standard’ for PFGE and the use of thiourea to achieve typeability of nontypeable isolates were described. The meeting also provided the opportunity for the nominees for the PulseStar awards for 2001 to be announced and the winners presented with their prizes by Dr. Swaminathan and Dan Cameron (see article 2001 PulseStar Awards later in this issue).

Rosemary Humes, APHL opened the 5th Annual PulseNet Update Meeting

The Friday morning sessions began with 'Talkback to PulseNet Too,' chaired by Carol Worthington from Tennessee Department of Health Laboratory Services. This was followed by presentations from various CDC branches and programs with activities related to PulseNet. Christine Steward and Linda McDougal of the Division of Healthcare Quality Promotion, described the *Staphylococcus aureus* project protocols and characterization of isolates currently in the national database. Susanna Schmink and Gwen Barnett from the Division of Bacterial and Mycotic Diseases, Meningitis and Special Pathogens Branch, presented the current status and imminent goals of *Neisseria meningitidis* and *Bordatella pertussis* PFGE. A session on CaliciNet was presented by Steve Monroe of the CDC Viral and Rickettsial Diseases Branch who described the molecular epidemiology of Norwalk-like viruses. The final presenta-

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Call For Contributions.

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)

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2001 PulseStar Awards

Three 2001 PulseStar awards were presented at the Annual PulseNet Update meeting to candidates who showed outstanding dedication and achievement in the year FY2000. From the six nominations received the winners were:

Stephen Dietrich, Laboratory Scientist, Michigan Department of Community Health, for his outstanding PFGE analysis (since 1989) and for being an integral part of the PulseNet team. Because of his expertise in the technical aspects of PFGE testing and image analysis, Michigan rapidly identified and reported several outbreaks during the previous year that proved to have an impact upon other states. Steve was also instrumental in drafting a standardized reporting format for the summarization of PulseNet data on a quarterly basis. He maintains an active interaction with CDC and other state laboratories that permits rapid exchange and dissemination of data.

Stacey Kinney, Microbiologist II (previously Senior Microbiologist) Connecticut Department of Health Laboratories, for her efficient and consistent communication with PulseNet and other laboratories through the PulseNet listserv. Stacey can always be relied upon to respond to a posting regarding regardless of the organism or if she has a match. She submits quality gels consistently and she represents a model PulseNet Laboratory, following protocols and naming gels and submitting correct lane information. The key to PulseNet is consistency and Stacey is an excellent example of this.

Laura Kornstein, Chief, Environmental Microbiology Laboratory, New York City Department of Health Communicable Disease Program, for playing a pivotal role in investigating foodborne diseases in New York City by overseeing PFGE subtyping and actively collaborating with PulseNet. In 2000, Dr. Kornstein was instrumental in identifying a multistate listeriosis outbreak when she noticed 12 clinical isolates of a rare ribotype DUP-1053 possessing indistinguishable PFGE patterns. Dr. Kornstein’s initial posting to the PulseNet listserv led to identification of cases in eight states. Subsequent investigations matching environmental isolates by ribotype and PFGE pattern with isolates from patients implicated deli meats and resulted in a large recall of contaminated products. This example highlights Dr. Kornstein’s ongoing contributions to PulseNet.

PulseNet would also like to thank the three runners up, **Robert C. Manning Jr.,** Clinical Laboratory Technologist, Georgia Division Public Health Laboratory , **Jennifer Adams,** staff member of the Minnesota Area Laboratory and **Wayne Chmielecki**, Chemist II, Pennsylvania State Public Health Laboratory for all their efforts and enthusiasm they demonstrated throughout FY 2000.

PulseNet Meeting -Continued from page 1

tion of the day was by Ian Fisher of the United Kingdom PHLS Communicable Disease Surveillance Center, who described the role of EnterNet-Europe in the international control of foodborne infections. The meeting was brought to a close on Friday at lunchtime, but not before an unscheduled announcement by John Threlfall of the PHLS. Dr. Threlfall stated that he was so impressed with the PulseNet scheme that on return to the UK he intended to make very strong recommendations for the establishment of PulseNet Europe and looked forward to further international collaboration between both sides of the Atlantic. The Michigan State Public Health Laboratory has offered to host the 6th Annual PulseNet update meeting.

FY 2000 Achievements

Laboratories participating in PulseNet at the end of 2000 included 46 state public health laboratories, the public health laboratories in New York City and Los Angeles County, the Special Projects and Outbreak Support Laboratory of USDA-FSIS, the FDA-CFSAN laboratory, FDA-CVM laboratory, and five FDA field laboratories. This represented an increase of 12 laboratories compared with the number of laboratories in PulseNet on December 31, 1999. Canada became the first international PulseNet participant with the formation of PulseNet North. The National Laboratory for Enteric Pathogens, Winnipeg, Manitoba, coordinates the standardized subtyping of foodborne pathogenic bacteria by PulseNet protocols in six provincial laboratories. CDC received 17,309 PFGE patterns in 2000; this reflected a 76% increase in PFGE pattern submission over 1999.

2001 PulseNet Goals.

1. Establish on-line databases for *E. coli* O157:H7, *Salmonella,* and *Listeria*.
2. Achieve 100% subtyping for *E. coli* O157:H7, and *Listeria monocytogenes*.
3. Establish real time (<48 h) subtyping for *E. coli* O157:H7 and *Listeria monocytogenes*.
4. Assure timely submission of all PFGE patterns of *E. coli* O157:H7, *Salmonella*, and *Listeria* to the PulseNet Server and all PFGE patterns of other foodborne pathogenic bacteria *(Shigella, Campylobacter jejuni* and *Vibrio)* to the PulseNet Database Administration Team at CDC.
5. Complete certification of 40 laboratories for *E. coli* O157:H7 and *Salmonella* and 30 laboratories for *Listeria.*
6. Begin distribution of PulseNet Newsletter by June 30, 2001.
7. Compile PulseNet Annual report for 2000 by June 30, 2001.

2001 New PulseNet Publications

Graves LM, Swaminathan B. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol 2001; **65**:55-62.

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol 2001; **39**:1889-94.

Swaminathan B, Barrett TJ, Hunter SB, Tauxe RV, PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 2001; **7**:382-9

2001 Abstracts

Kubota KA, Hunter SB, Barrett TJ, Ackers ML, Mintz ED, Analysis of *Salmonella* serotype Typhi pulsed-field gel electrophoresis (PFGE) patterns associated with international travel. 101st American Society for Microbiology General Meeting, Orlando, Florida 21 - 24 May 2001.

Van Duyne MS, Hunter SB, Evans MC, Barrett TJ, Holland B, Torres P, Abbott S, Pallipamu R, Pride K, PulseNet Participating Laboratories. Genetic diversity of *Salmonella* Poona: the PulseNet experience. 101st American Society for Microbiology General Meeting, Orlando, Florida 21 - 24 May 2001.

Zhao S, White DG, Datta A, McDermott P, Friedman S, English L, Ayers S, McDermott SD, Wagner DD, Walker RD, Characterization of *Salmonella* obtained from animal derived dog treats in the United States. International Conference on Emerging Infectious Diseases, Atlanta, Georgia 16-19 July, 2000.

Recent *E. coli* Outbreaks in Michigan

Michigan experienced two simultaneous outbreaks of *E. coli* O157:H7 infections in March of this year. Ten cases with specimen collection dates during mid-late March were identified. PFGE revealed two clusters with unrelated patterns plus two cases with patterns unrelated to the clusters.

Outbreak A consisted of five cases in which specimens had indistinguishable *XbaI* and *BlnI* patterns that were new to the Michigan database. The cases were linked to home-prepared ground beef purchased at different grocery stores. Traceback investigation revealed a likely common source of meat. Additional cases with the outbreak pattern were identified in Wisconsin and Illinois. The USDA located and tested warehoused samples of the epidemiologically implicated ground beef; culture of one sample grew *E. coli* O157:H7. This investigation led to the recall of ground beef products from a meat plant in Milwaukee, Wisconsin.

Outbreak B consisted of three cases that were identified in late March and a later case identified in mid-April. Specimens from these cases had indistinguishable *Xba*I and *Bln*I patterns that were unrelated to the patterns of Outbreak A and were also new to the Michigan database. These cases also were linked to home-prepared ground beef; the meat was purchased at different locations of a grocery chain. Isolates with the outbreak patterns were cultured from an opened package of ground beef from the freezer of one of the case-patients.

These outbreak investigations were aided greatly by the use of PFGE. PFGE indicated that two unrelated outbreaks had occurred rather than one larger outbreak, and epidemiologic evidence later supported this. Two temporally associated cases were eliminated from the investigation because of their unrelated PFGE patterns, further helping to focus the investigation.

By Stephen E. Dietrich,
Michigan Department of Health, Molecular Biology Section

Cost of PFGE: *An Estimate*

One of the most frequently asked questions about PFGE is how much does it cost? While this question is simple, the answer to it is more complicated than it appears. We have come up with estimates on the cost of PFGE (not to be confused with the cost of PulseNet). The figures found in Tables 1 and 2 address the cost of PFGE from the time a culture(s) is received to the time an image of the gel containing that culture(s) is captured. Data management and database comparisons were not included in these calculations. The estimates are based on what a typical CDC laboratory would expect to pay for the reagents and supplies needed to perform PFGE.

Table 1. Cost of PFGE reagents per sample for PulseNet Protocols

Reagent	Amount Required/Test	Cost/test	
SeaKem agarose Plug Running gel	 0.5 ml/plug 10 isolates/gel	 $0.02 $3.44/10=$0.34 **Total = $0.36 / sample**	
TE buffer	65 ml/sample (needed for plug agarose/washes) Commercial source From scratch	 $0.90 $0.02	
Proteinase K	50 ul/plug	$0.90	
Lysis Buffer	5 ml/plug	$0.35	
Restiction enzyme*: *Xba*I *Bln*I (AvrII) *Spe*I	 50 Units/plug slice 25 Units/slice 25 Units/slice	 $1.00 $7.50 $6.80	
Restriction buffer H**	40 ul/plug slice	$0.20	
TBE buffer	2,200 ml/gel	$1.50	
Totals: With *Xba*I With *Bln*I With *Spe*I		Commercial $ 5.21 $11.71 $11.01	"Scratch" $ 4.33 $10.83 $10.13

*** Cost of other enzymes used for PFGE:**

1. *Sma*I: 40 units/slice; ~$5.00/slice
2. *Asc*I: 25 units/slice; ~$2.50/slice
3. *Apa*I: 200 units/slice; ~$2.50/slice
4. *Kpn*I: 40 units/slice; ~$1.00/slice

**Need to purchase additional buffer; not enough buffer is provided with the enzymes

Table 1 lists the cost of reagents needed to prepare PFGE plugs and to run the agarose gels. While the cost of enzymes varies from vendor to vendor, in general, the cost of the enzymes listed in Table 1 is close to the average price provided by the main suppliers in the United States. This is also true for most of the other reagents listed in this table.

It is estimated that it would take the average laboratory person 3.5 hours to process 1-5 samples (plugs) or 5.5 hours to process 10-15 samples. The actual time required may vary from laboratory to laboratory, depending on experience and the set-up of the laboratory. In this table the cost of labor was calculated based on a salary range ($10, $15, $20, $25, and $30 per hour) that was inclusive and representative of the staff found in most PulseNet laboratories.

By Efrain Ribot, Chief of PulseNet Methods Developments and Validation Laboratory, CDC

Related article, Elbasha EH, Fitzsimmons TD, Meltzer MI. Cost and benefits of a subtype-specific surveillance system for identifying E coli O157:H7 outbreaks. Emerg Infect Dis, 200;293-297. This article can be downloaded from the CDC website.

Table 2. Estimated total labor-reagents costs per sample

Salary ($/h)	Total labor cost per 10-15 samples	Total cost of reagents and labor per 10 plugs	"Theoretical" cost of reagents and labor per plug (value in previous column divided by 10)	Total labor cost per plug (1-5 samples)	Total cost of regents and labor per plug
$10.00	$55	$105-165	$10.50-16.50	$35.00	$40.00-46.00
$15.00	$82.50	$132-192	$13.20-19.20	$52.50	$57.50-63.50
$20.00	$110.00	$160-220	$16.00-22.00	$70.00	$75.00-81.00
$25.00	$137.50	$187-247	$18.70-24.70	$87.50	$92.50-98.50
$30.00	$165.00	$205-275	$20.50-27.50	$105.00	$110.00-116.00

Table 2 lists the total cost of PFGE. The table also contains a column with "theoretical estimates" of the cost of PFGE per plug. The theoretical values provide a cost figure that is easier to understand, although these values can be considered an under-estimate of the actual cost per PFGE plug. This is because the time required to process a single sample is not significantly different from that needed to process 10-15 samples. In other words, processing one sample does not take one tenth of the time needed to process 10 samples. The more accurate estimates can be found in the shaded columns 3 and 6 of this table. The cost of PFGE can be reduced by pooling isolates when possible. For instance, the plug preparation steps of the *E. coli* O157:H7, *Salmonella*, or *Shigella* protocols are the same, allowing one to pool isolates from these organisms. Once the plugs are made, they can then be restricted and electrophoresed under the appropriate conditions. This will reduce "hands on time" significantly, which is the most expensive aspect of PFGE. However, pooling of isolates should be done in a way that does not compromise the goal of achieving real-time subtyping status for *E. coli* O157:H7.

Finally, it is very important to view the cost estimates of PFGE in the context of public health. A wealth of information attests to the usefulness of PFGE as a molecular tool for early detection of clusters of foodborne illness. Early detection and prevention of illness significantly reduce cost of health care and potentially the loss of lives, which are much higher prices to pay.