Certification and Proficiency Testing: Where We Stand Now
Christine Stewart, APHL Contractor, PulseNet QA/QC Program

The PulseNet Quality Assurance/Quality Control (QA/QC) Program, which includes certification and proficiency testing of the PulseNet participating laboratories, is growing. The purpose of the program is to allow accurate comparisons of TIFF images within the PulseNet national databases by ensuring that TIFF files of all gel images are comparable, of excellent quality, and have correct normalization and consistent band assignment.

Certification. Currently, certification is a one-time event for each person for each PulseNet organism. Individuals can be certified for “TIFF image only” (i.e., laboratory methods for Pulsed-field gel electrophoresis [PFGE] and image acquisition), “analysis only” (i.e., BioNumerics analysis of gels using PulseNet client scripts and access to the national database), or “TIFFs and analysis”. To become certified, laboratorians should send an email to PFGE@cdc.gov requesting the strain set that they wish to have for certification. The number of strains in each set was reduced in 2003 so that only one 10-well gel is required; PulseNet-related activities should be certified. Laboratories with analysis-certified individuals can query and upload their own data to the national databases.

In the near future, a group of individuals from PulseNet-participating laboratories will help create a document detailing a change in procedures for submission of TIFF images within PulseNet national databases. The SecurID key fob login for on-line access to the national database. The SecurID key fob is assigned to an individual, rather than a laboratory, and thus must only be used by that assigned (certified) individual.

Currently, CDC can provide a maximum of two SecurID key fobs to each certified PulseNet participating public health laboratory, even though there may be more than two certified people in a laboratory.

As of 2003, more than 150 individual certifications were completed, an impressive number of U.S. PulseNet-participating laboratories with individuals who are certified in analysis for E. coli O157:H7 (41 laboratories), Salmonella (37 laboratories), and Listeria (14 laboratories). However, these numbers do not include all U.S. participating laboratories. Compliance with certification procedures is a requirement for all PulseNet participating laboratories; therefore, all laboratorians working in

and/or bundle certification files are emailed from the participating laboratory to PFGE@cdc.gov. We then evaluate the submissions and report back to the submitter. If the submitter is deemed analysis-certified, the individual will receive a SecurID key fob and login for on-line access to that database. The SecurID key fob is assigned to an individual, rather than a laboratory, and thus must only be used by that assigned (certified) individual.

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(certification of laboratories instead of individuals) to the current method of certification. The goal is to streamline the certification process and encourage participating laboratories to be responsible for the continuing quality of all gels produced in-house. In order to maintain TIFF responsibility for the continuing quality of all gels and encourage participating laboratories to be goal is to streamline the certification process

1) Satisfactorily complete annual proficiency tests.
2) Establish an in-house QA/QC program to verify that individuals are following the standardized protocol and are producing quality work.
3) Undergo an annual review of their QA program by their area laboratory.

Proficiency Testing. Certification is valid as long as the laboratory successfully completes annual proficiency testing. For proficiency testing, one strain per PulseNet organism is sent to laboratories with individuals certified in analysis. Half of the certified laboratories receive proficiency test strains in the Fall and the other half receive strains in the Spring. Participants produce an in-house gel, analyze and upload it to the proficiency test database. This year a new component, “TIFF sent by CDC,” was added to the most recent round of proficiency testing. For the new component, all laboratories analyze the TIFF sent by CDC and submit the results for each organism in which they are certified. This component is completed along with the in-house gel production and in-house gel analysis as part of the proficiency testing survey. The “TIFF sent by CDC” component was instituted at the suggestion of participating laboratories as a control for gel-to-gel variation affecting band marking from laboratory to laboratory.

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 pertaining to the above, please include the words “Certification” or “Proficiency Testing” in the subject line. Our goal is for all participating laboratories to be on-line. This will make comparisons with the national database more efficient for all laboratories and will further speed the identification of outbreaks.

Real-Time Subtyping (Continued from page 1)

recently decreased, a number of outbreaks were identified in the past two years. Running both enzymes for L. monocytogenes is not time-consuming because we usually receive only one isolate at a time. In addition, strains of any organism occurring in clusters or outbreaks under investigation are run with a second enzyme as soon as possible.

Epidemiologists sometimes delay starting an investigation until matches are confirmed with a second enzyme, which may adversely affect the discovery of an outbreak source. Results of second enzyme testing are important for epidemiologists so that some cases that are less likely to be outbreak-related can be excluded from their investigations. Excluding such cases can increase the accuracy of analyses performed. Many outbreaks involve a fairly small number of cases in any state; therefore, we need to optimize the epidemiologic specificity of our testing. For example, cases with primary enzyme patterns differing only slightly from an outbreak pattern are sometimes included in epidemiologic investigations. The second enzyme testing will allow epidemiologists to better discern pattern differences.

Second enzyme testing is probably more important for primary enzyme patterns that are found more commonly. For instance, we have found one XbaI pattern in 31 isolates of E. coli O157:H7 identified since 1999; 11 BlnI patterns were found in 24 (of 31) isolates tested with this enzyme. The above table describes the compiled data on the discriminatory ability of the second enzyme for Michigan’s isolates of E. coli O157:H7 and O157:NM, L. monocytogenes, and S. Newport. For each organism, the second enzyme proved to be very good at subtyping primary enzyme clusters. Discrimination occurred for temporally-similar and dissimilar clusters.
PulseNet Training Workshop for Food Industry Scientists

The New Mexico State Lab is located in Albuquerque on the University of New Mexico campus. It is officially known as the Scientific Laboratory Division (SLD) of the New Mexico Department of Health (NMDOH). It houses three Bureau (Toxicology, Chemistry and Biology) as well as the New Mexico Department of Agriculture Veterinary Diagnostic Services - VDS, and The University of New Mexico, Office of the Medical Investigator - OMI. The Biology Bureau includes six sections: General Microbiology, Environmental Microbiology, TB/Mycology, Virology/Serology, Metabolic Screening, and Molecular Biology. These sections handle various types of samples including clinical hospital specimens that are sent for confirmatory identification and serotyping, public health clinical samples; VDS and OMI samples; environmental samples such as milk, food and water; and bloodspot newborn metabolic screening samples. The laboratory serves as a support service to the Department of Health, Department of Agriculture and the Environmental Department.

The SLD uses Microsoft Access as a LIMS system to track samples from all sources and sections. As samples enter the building through the specimen receiving area, they are sorted as the demographic and specimen information is entered into the database. The sample is then transferred to the appropriate section and processed. At the Scientific Laboratory Division (SLD), two laboratory scientists are trained in PFGE. Both laboratory scientists process samples, make plugs, and run gels. Only one scientist, the PFGE technician, is in charge of analyzing gels and reporting data which ensures consistency of data analysis.

An enteric sample submitted to the General Microbiology Section is typically identified to serotype. Several scenarios may occur that will result in the identification of an organism implicated in a foodborne outbreak:

1) In the event of multiple samples being identified with the same serotype, the laboratory scientist working up the samples will communicate this information to the PFGE technician.

2) Sometimes, the Office of Epidemiology is informed by doctors or hospitals of a cluster of patients who exhibit similar symptoms. The Office of Epidemiology will then contact the PFGE technician and the General Microbiology section supervisor to inform them of the potential for epidemiologically connected samples. This sharing of information facilitates the work-up performed by the laboratory scientists.

3) On most occasions, an outbreak is detected only after several weeks have elapsed because samples come in sporadically and laboratory scientists do not notice a significant increase in a particular serotype.

4) Lastly, if a rare serotype is isolated a number of times within a short period of time, the PFGE technician will be asked to run those isolates prior to routine PFGE work.

The resulting PFGE patterns are examined rigorously to identify any trends of epidemiological significance. For each sample, the pattern designations are input along with any other results into the Access database. This allows the user to search the database for previous isolates with the same pattern designation, serotype, submitter, dates of collection and receipt. Also, BioNumerics is used to analyze gels and produce dendrograms, the Access database is used to query for other samples that had the same pattern designation. The data from such a query provides all the information from each sample, as opposed to the information found in the BioNumerics window where only some of the data fields are present in an observable view. Also, the Access database is used to create reports that are sent to both CDC and the Office of Epidemiology.

If a serotype cluster results in identical pattern designations, the Office of Epidemiology is immediately informed. The Office of Epidemiology was recently alerted when a rare Salmonella serotype was identified (Kambio) from beef jerky packaged in New Mexico. Early in the suspected outbreak, all new samples of the same serotype coming into the lab were run with priority on PFGE.

In addition, we notified CDC PulseNet via the PFGE mailbox and posted on the WebBoard to see if this pattern was identified in any other states. The Office of Epidemiology interviewed every patient from whom samples had been obtained, but at the beginning of the investigation were unable to pinpoint a single source of infection. Back at the laboratory, a second restriction enzyme was used in the PFGE analysis to see if the presumed single source of infection was possibly multiple sources. We obtained no greater discrimination of the patterns from the new analysis. The Office of Epidemiology conducted more interviews with patients, and finally determined a single food source associated with the infection.

The food source was beef jerky.

The Office of Epidemiology continued to work with other state and federal agencies including the Albuquerque Environment Department, USDA/FSS, and FDA. Beef jerky samples arrived at the laboratory and were processed by the Environmental Microbiology Section to isolate any potential foodborne pathogens. Stool samples from people who worked at the beef jerky processing plant were sent to the General Microbiology Section. If Salmonella Kambio was isolated, PFGE was run immediately to corroborate the epidemiological investigation. The outbreak was identified within a short period of time, and the Office of Epidemiology followed up with patients.

Since the NM DOH Scientific Laboratory Division processes clinical, veterinary, and OMI samples from the entire state, the laboratory has a unique role in the detection of disease outbreaks and surveillance in New Mexico. The statewide picture of New Mexico’s health can be observed from a centralized location and the excellent communication between the laboratory and the Office of Epidemiology facilitates timely outbreak detection and rapid response.

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The 6th International Meeting on Microbiological Epidemiological Markers (IMMEM6) was held at the winter resort of Les Diablerets in Switzerland from August 23-26, 2003. The IMMEM is held every three years and all six meetings thus far have been held in Europe. The IMMEM focuses entirely on typing and typing methods for infectious disease agents, with particular emphasis on the epidemiology of human and veterinary pathogens. Because of the narrow focus, the IMMEM is usually a small meeting with attendance in the 100 to 200 range, thus maximizing interactions and stimulating discussions among the delegates. This year, IMMEM6 had a record attendance of 350 delegates.

Presentations at previous IMMEMs provide a chronological history of the evolution of molecular typing methods. Typing methods in routine use today, such as plasmid profiling, microrestriction analysis, ribotyping, pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, amplified fragment length polymorphism, and multilocus sequence typing were introduced to many of the participants for the first time in previous IMMEMs.

High throughput Single Nucleotide Polymorphism (SNP) analyses and Multi Locus Variable number tandem repeat Analysis (MLVA) were the two newer typing approaches highlighted at IMMEM6. The SNP and MLVA typing methods require prior knowledge of sequence variation at specific genomic loci. Each SNP analysis detects polymorphisms at a single locus on the genome, and the determination of SNPs at multiple loci may provide sufficient information for strain typing. SNP analysis may be considered a simplified version of MLST, which catalogs nucleotide differences in the sequences of seven or more housekeeping genes. However, data acquisition for MLST is more time extensive, as one needs to sequence the fragments of the housekeeping genes under study. In contrast, for SNP-analysis one needs to detect the sequence variation only at a single nucleotide position; this may be indirectly assessed by real-time PCR with concurrent melting point determination of the PCR-fragment or by using DNA arrays. PM Giffard from Australia described a software program which could identify informative SNPs from public domain MLST-databases. The N. meningitidis and the S. aureus databases were used as examples to show how the informative SNPs could be used to identify the different sequence types in the databases or offer the best discrimination between unrelated isolates.

While mutations in intergenic regions do not affect the amino acid sequence of genes, a mutation could affect gene regulation and therefore an iSNP may be under selective pressure.

Often, MLST and MLST-based SNP-analysis do not provide adequate discriminatory power for outbreak investigations; they are most useful for phylogenetic analyses or to track long-term epidemiological patterns. However, SNPs in regions outside the housekeeping genes may be useful to study short term relationships between strains, i.e. to identify outbreak strains. J Musser, USA, gave an overview of SNP-analysis and its utility for epidemiological studies. Synonymous SNPs (sSNPs) are SNPs that occur in genes but do not result in amino acid changes when translated. These are useful for inferring the phylogenetic relationships between closely related strains. Non-synonymous SNPs (nsSNPs) are SNPs that result in amino acid changes when translated, thus resulting in an altered protein sequence and possibly also in altered protein function; for this reason, nsSNPs are often under selective pressure and therefore are not reliable for phylogenetic analysis. A special variant is the iSNP, which is an SNP in an intergenic region. The utility of this type of SNP may be similar to nsSNP. While mutations in intergenic regions do not affect the amino acid sequence of genes, a mutation could affect gene regulation and therefore an iSNP may be under selective pressure.

The MLVA method has been discussed in previous issues of PulseNet News and is under development as second generation PulseNet methods for E. coli O157, Salmonella, and Listeria monocytogenes.

WP Terletski from Germany introduced a method he termed Subtractive Restriction Fingerprinting. This is basically a variation of microrestriction analysis. Purified DNA is digested using two enzymes; then two oligonucleotides that match the recognition sequence of each restriction enzyme are labelled with biotin or Dig-UTP, and are ligated to the restriction fragments. The fragments now labelled with biotin are removed by the use of streptavidin coated beads, leaving only the fragments labelled with Dig-UTP in both ends in solution. These are then separated in a gel and visualized using standard Dig-visualisation technology. In this way the resulting fragments show equal intensity because (Continued on page 6)
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maintained by Dr. Martin Wiedmann’s Laboratory of Food Microbiology and Pathogenesis of Foodborne Disease.

In his introductory remarks, Dr. Bala Swaminathan, Chief, Foodborne and Diarrheal Diseases Laboratory Section, CDC, identified the establishment of key partnerships as the next frontier for PulseNet. A partnership between PulseNet and the food industry could potentially allow industry access to current information on prevalent DNA fingerprints of pathogenic strains involved in human disease, facilitate the establishment of monitoring programs by the food industry for outbreak associated strains, and improve the industry’s ability to assess the likelihood of involvement of specific food processing plants in outbreaks under investigation based on comparisons of outbreak pattern(s) with plant monitoring data. If obstacles to this kind of collaboration can be overcome, the public health sector could benefit from information from the food industry on the association of specific subtypes of foodborne pathogens with specific food types, particularly in early stages of outbreak investigations. This collaboration and exchange of information would improve the ability of public health agencies to assess the effectiveness of control and prevention programs for foodborne diseases. CDC

Students hard at work!

PulseNet Database Development: Progress and Plans

The PulseNet Database Development team provides the following update on efforts to improve the PulseNet databases and client scripts. Overall, recent efforts have been focused on 3 goals:

1. Development of client scripts and on-line database for Shigella.
2. Transitioning the E. coli database to SQL format.
3. Changing to a master script format for PulseNet scripts.

Progress on each of these goals impacts the progress made on the others.

During the development of the Shigella scripts and the conversion of the national Shigella database to the on-line format, we decided to create the database in SQL format. The database team is testing the SQL-formatted database at this time. The scripts should be ready for first round testing very soon.

The first step in formatting the E. coli database to SQL was converting the current database to a SQL test system. The second step was testing a new script for uploading data to a SQL format database. The PulseNet Database Development Team thanks the volunteers who have tested various versions of this script and instructions for installation and use. The new scripts allow the user to upload multiple lanes from a single TIFF to a SQL-formatted national database. This improvement will allow quicker uploading to the national database.

Another improvement is the transition to a master script format for scripts development and distribution. All client scripts will become part of a master script set with modifications as needed for specific organisms. This will allow the development team to more uniformly upgrade all client script sets. The master script format will also be more cost effective for future improvements. Many of the improvements that were/will be added are due to requests from the PulseNet participants. Please continue to let us know what changes and additions you would like to see. Also, let us know when you encounter errors or problems by sending an email to PFGE@CDC.GOV or calling (404) 639-4558. By improving the client scripts, we can make the national data easier to access and work with.
the band intensity is only dependent on the copy number of fragments and not on the size of the fragment as in an ethidium bromide stained gel. With careful selection of restriction enzymes up to 50 fragments may be visualized on a gel after ordinary electrophoresis. The enzyme combination EcoRI-PauI was found suitable for the typing of zoonotic salmonella serotypes. The entire procedure up to electrophoresis is completed within 4 hours and the method compares favourably with PFGE in this respect. However, because restriction-based typing methods are being replaced by amplification and sequencing-based methods, this method is unlikely to get the recognition it might have achieved, had it been invented 10 years ago.

Despite the high level of interest in new subtyping methods, a workshop on PFGE drew a capacity crowd at the meeting. Dr. Richard Goering and Dr. Marc Struelens led the discussions and responded to questions and comments from the audience. PulseNet protocols were frequently mentioned in the discussions by PulseNet meeting organizers because this will give an opportunity for cross-fertilization of ideas and subtyping strategies between the two groups.

Publications and Abstracts

October 2003. Nehal graduated from the University of Georgia in August 2003, with a B.S. in Biology. She will be working on a project funded by USDA, involving the analysis and data management of Salmonella PFGE patterns.

Beth McGlinchey, a USDA employee, will be working in conjunction with the PulseNet database administration team to create and establish a Salmonella database of PFGE patterns from USDA isolates. Beth attended Georgia College and State University, receiving her B.A. in 2001 and her M.S. in Biology in May 2003.