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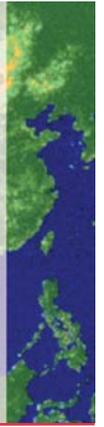


The National Molecular Subtyping Network  
for Foodborne Disease Surveillance



# PulseNet News™

State & Local Public Health Laboratories  
in the United States



## PulseNet Latin America - Expanding the Network

The PulseNet program started in Latin America (LA) three years ago and has grown to develop increasingly deep roots. The principal goal is to establish a network of laboratory based surveillance of foodborne diseases (FBD), similar to those already existing in other parts of the world, in order to improve the diffusion of microbiological information on the safety of the food products. PulseNet, the molecular subtyping network for FBD surveillance created by the United States Centers for Disease Control and Prevention (CDC), supports PulseNet LA by means of technology transfer, training and external quality control.

Other institutions in LA that have played an important role in the creation and continuous strengthening of PulseNet are Pan-American Health Organization (PAHO) and Instituto Nacional de Enfermedades Infecciosas (INEI) -

ANLIS "Carlos G. Malbrán." While INEI is in charge of the technical support regarding PFGE protocols, analysis, certification and quality control programs, PAHO provides all the aspects needed for the communication among members, server development and maintenance for the regional databases and project development. Both organizations, together with CDC, share responsibilities for strategic planning and performance of PulseNet LA.



Due to an increase in commercial trade between countries and the movement of people around the globe, foodborne infections represent nearly 70% of the cases of acute diarrheal disease in the world according to the data of the World Health Organization (WHO). Traditional bacterial pathogens that are involved in FBDs are *Salmonella* spp, *Shigella* spp, *Vibrio cholerae*, *Clostridium botulinum*; other emerging pathogens include *Escherichia coli* O157, *Listeria monocytogenes* and *Campylobacter jejuni*.

In this context, the main objectives of the PulseNet LA network are to strengthen national and regional surveillance of FBDs; determine the burden of disease; detect early emerging and reemerging pathogens; establish national

and regional databases; strengthen communication among the member countries; and actively use the national and regional information in coordinated public health actions and interventions. Actions such as these are used to consolidate the collaboration, which involve not only clinical laboratorians but also epidemiologists. 



**All articles have been contributed by PulseNet Latin America**



Participants of 2006 PulseNet Latin America Annual Meeting

## 2006 PulseNet Latin America Annual Meeting

The fourth and most recent meeting of PulseNet Latin America was held in June 2006, in the INEI - ANLIS "Carlos G. Malbrán" in Buenos Aires, Argentina. The meeting discussed, among other subjects, laboratory protocols for PFGE subtyping of pathogens of food transmission. Discussions involved the progress and the difficulties experienced in each country, as well as operative issues of the network.

In the meeting, the plan of action for PulseNet Latin America was launched for the biennium 2006/07.

Some of the principal actions were:

- Foster communication between members of the network
- Carry out international research projects focused on the analysis of the genetic diversity of *S. Typhi*, *S. Typhimurium* and Shiga toxin producing *Escherichia coli* (STEC) circulating in the Region
- Develop national projects based on integrated surveillance of the food chain—including epidemiologic, laboratory, environment and veterinary aspects
- Obtain the certification of the regional center for *Listeria monocytogenes* and *Campylobacter jejuni* (these goals have already been achieved)
- Obtain the certification of national reference laboratories for gel performance (those who have not completed it yet) and for analysis (those who have the BioNumerics or Gel Compar II software)

- Provide additional training for the members of the network on specific topics, via Internet, with materials provided by CDC
- Send support missions for countries such as Cuba, Nicaragua, Colombia, Peru, Venezuela and Uruguay.

PulseNet Latin America sees the subtyping of the following microorganisms as a priority for the region: *Salmonella* spp.; all *E. coli*; *Shigella* spp.; *Listeria monocytogenes*; *Campylobacter* spp.; *Vibrio cholerae*; and *Vibrio parahaemolyticus*. Some of the principal challenges are to incorporate, analyze and use the results of PulseNet. Efforts must also be made to coordinate the information and actions among the Latin American countries as well as with other related FBD networks of Latin America; and actively participate in the PulseNet international network.

The following activities are being performed in the region:

- Application of standardized and validated subtyping protocols
- PFGE analysis with software using PulseNet scripts
- Creation and maintenance of national and regional databases
- Quality program for the network (periodic certification and proficiency tests)
- Communication (meetings and conferences)
- Exchange of information
- Continuous training
- Gradual incorporation of foodborne pathogens taking into account the needs of the network's member countries. **CDC**

## Development of PulseNet Latin America: 2003 to 2006

Three years ago, the first step towards the establishment of PulseNet Latin America was taken. Country representatives, in both microbiology and epidemiology fields, from Bolivia, Brazil, Chile, Costa Rica, Cuba, Nicaragua, Argentina, Mexico, Paraguay, Peru, Venezuela and Uruguay, as well as professionals from the CDC and PAHO, created the network together. The following year (2004), saw the official integration of seven countries—Brazil, Chile, Colombia, Mexico, Argentina, Uruguay and Venezuela, and the program was formalized with the approval of a Memorandum of Understanding between the countries. In addition, the first laboratory and BioNumerics software training course was held. During the 2005 meeting, six more countries were incorporated into the network (Bolivia, Costa Rica, Cuba, Nicaragua, Paraguay and Peru), and an additional training course took place.

The network meetings in 2004, 2005 and 2006, whose highlights are presented in the next pages, were held in Buenos Aires, Argentina with microbiologists and epidemiologists from all the participating countries present. **CDC**



Swami, Isabel Chinen, Molly Joyner, & Peter Gerner Smidt in Buenos Aires, Argentina

## Training Workshops for Latin-American PulseNet Participants

For three consecutive years, Buenos Aires was the chosen location for the PulseNet Latin America training workshops. The first of these courses, held in July 2004, strengthened the commitment to organize and develop the Latin American network. During four days, the instructors included Norma Binsztein, Marta Rivas, Mariana Pichel, Isabel Chinen (INEI); Bala Swaminathan, Efrain Ribot, Jennifer Kincaid, Paola Bordoni (CDC), John Besser (MN), and Enrique Perez (PAHO); representatives of the first seven member countries discussed and practiced the standardized protocols of PFGE for *E. coli* O157: H7 and *Salmonella* spp.; exchanged experiences and clarified concerns on the main laboratory techniques linked to food safety.

In 2005, with the incorporation of Bolivia, Costa Rica, Cuba, Nicaragua, Paraguay and Peru to the network, the workshop achieved even better results. Five days of intense work were devoted to the following: practical PFGE training, presentation of new subtyping methods, and issues linked to quality control. The objectives and technical aspects related to the use of the BioNumerics software program were also revised and discussed. The same faculty staff as in 2004 participated in this workshop, with Kelley Hise, from CDC, instead of Jennifer Kincaid.

The most recent meeting, carried out June 20th and 21st 2006, was preceded by a software workshop, where BioNumerics was discussed in detail and compared to Gel Compar II. The training staff from CDC included Bala Swaminathan, Peter Gerner-Smidt, Nehal Patel and

Molly Joyner. The continued increase in the number of participants and their related training shows that PulseNet Latin America is on solid ground and is in full expansion mode. Also, helping the network's consolidation were the technical cooperation visits made to member countries: in 2005, to Venezuela and Uruguay and in 2006, to Mexico and Peru. Though challenges are many, the goal for PulseNet LA remains to grow, disseminate and increase the knowledge about surveillance and control of foodborne diseases.

CDC



## Colombia Surveillance to Control Foodborne Diseases

At the third annual PulseNet Latin America meeting in June 2005, the Microbiology group of the Instituto Nacional de Salud (INS) of Colombia presented an analysis of the country's surveillance network in the control of foodborne diseases (FBD), with data collected from 1997 to 2004. The Andean nation has a total of 138 clinical laboratories. Public health laboratories are present in 24 of the 33 Colombian states.

In the seven year period from 1997-2004, the surveillance network of Colombia isolated 60 serotypes of *Salmonella* spp. Of these 1,531 isolated microorganisms, 32% were Typhimurium, 30% Enteritidis, 6% Typhi and 32% other serotypes. The INS initiated the genetic characterization of *Salmonella* and also created a national database with the PFGE subtypes of the microorganisms isolated in 19 states.

With knowledge of the PFGE patterns, the Colombians could identify clusters of related strains and confirm outbreaks; avoid unnecessary studies; detect genetic relationships that could not be discovered by the traditional methods of surveillance and control the source and transmission of infections. The PFGE patterns identified in Colombia will be integrated into the Latin America PulseNet Database. CDC



## Take a tour of the Network

From each one of the countries that participate in PulseNet Latin America, at least one National Reference Laboratory is the laboratory member in the network.

### Chile -

The Instituto de Salud Pública has been performing genetic characterization of Chilean strains of enterohemorrhagic *Escherichia coli* (EHEC) since 1980. Since 2000, all strains of ShigaToxin producing *E. coli* (STEC) from clinical and non-clinical isolates (food, environmental, and animal) are confirmed by the VERO cell assay. The genetic characterization is very useful in the epidemiological surveillance, which includes Hemolytic Uremic Syndrome (HUS). PFGE has already been used in Chile for the subtyping of this pathogen.

### Mexico -

The Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), presented the country's progress in the implementation of PFGE for *Salmonella* spp. In 2006, nine professionals at InDRE were trained (three from the Laboratory of Molecular Bacteriology and six from the laboratory in charge of

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## Tour of the Network

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isolation, biochemical and serological identification of *Salmonella* spp). Between May and June, a course in molecular diagnosis of pathogens caused by ingestion of food, directed toward the personnel of the state laboratory network and coordinated by InDRE took place with the support and assistance of PAHO and WHO.

### Venezuela -

The principal goals of PulseNet in this country have been to integrate the laboratories currently trained in PFGE analysis and in food control in an epidemiologic pilot research project. The Instituto Nacional de Higiene Rafael Rangel intends to strengthen the relations with the Ministry of Health (Division of Control and Food Hygiene), with the Bureau of Epidemiological Surveillance and also with the regional laboratories. The institute has investigated cases of foodborne disease using the PulseNet PFGE protocols, including an outbreak of salmonellosis traced back to contaminated white cheese.

### Uruguay -

The Departamento de Laboratorios de Salud Pública presented a research project about the antibiotic resistance of enteric *Salmonella* serotypes. The institution has recently implemented PFGE and will apply this methodology for the genetic characterization of *Salmonella* spp. and STEC.

### Bolivia -

In Bolivia, the Instituto Nacional de Laboratorios de Salud has not yet implemented PFGE; however, it is currently working to integrate 63 national laboratories, from food and clinical areas. This will make it possible to disseminate the goals of PulseNet to the National Laboratory Network for future studies on *Salmonella*, *Shigella*, *Campylobacter*, STEC and *Vibrio cholerae*. Maintaining a constant partnership with the National Service of Epidemiology is another goal, as well as organiz-

-ing the information flow with regards to FBDs that includes hospitals and medical centers.

### Peru -

Peru is currently submitting proposals to expand its participation in PulseNet Latin America. Information about PulseNet has been disseminated via a Bulletin and the Magazine of Experimental Medicine of the Instituto Nacional de Salud (INS). The website of the INS ([www.ins.gob.pe](http://www.ins.gob.pe)) also publishes information about the network. The 24 laboratories that make up the network of the National System of the INS have contributed, as well as the epidemiology network of the Peruvian Ministry of Health to the diffusion of PulseNet in Perú. The INS has implemented PFGE, although it still lacks software for the interpretation of the results of FBD studies using PFGE.

### Paraguay -

The Laboratorio Central de Salud Pública is the organization which coordinates the network in Paraguay. Among the proposals for the development of the network is a study to look at the genetic diversity among the strains of *Salmonella* Enteritidis isolated in the last five years in Paraguay. Paraguay includes as part of the national PulseNet network: the Hospital of Clinics, the Institute of Tropical Medicine, Social Security Institute, the Baptist Medical Center, the Laboratory Diaz Gill and the Laboratory San Roque, and the National Hospital of Intagua. Seven more cities will be integrated in the near future.

### Cuba -

The National Reference Laboratory of Acute Diarrheal Diseases of the Instituto de Medicina Tropical Pedro Kourí, presented their works using Guefast 06, a system of mini-pulse field electrophoresis equipment (mini ECP) developed in Cuba. This system allows the fast acquisition of molecular pulsetypes of microorganisms. Guefast 06 is offered with protocols and sets of reagents for the non-enzymatic preparation of samples of intact and immobilized DNA. The compatibility of this equipment with the standardized PulseNet PFGE protocols will be evaluated.

### Costa Rica -

The Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (Inciensa), a public institution linked to the Ministry of Health, is responsible for coordinating surveillance of infectious diseases and became a PulseNet member in 2005. To date the institute has not worked with PFGE. Inciensa intends to begin with the characterization of *S. Typhimurium*, the principal agent of FBD in the country, and then to continue with the characterization of *Shigella flexneri*. Until Inciensa gets the necessary equipment, strains isolated in Costa Rica are being analyzed by PFGE at the Regional Technical Center (INEI). The results of a comparative study on *Salmonella* Typhimurium were presented at the last PulseNet update meeting in Miami, 2006.

### Brazil -

In the fourth meeting of the PulseNet network in Latin America, Brazil reported the experience of the Instituto Adolfo Lutz, linked to the Ministry of Health of the Government of the State of São Paulo, in the use of the standardized protocol of PFGE for *Salmonella* Enteritidis. The Institute is the national reference laboratory for *Escherichia coli* and the Brazilian regional reference for *Salmonella*, *Shigella* and *Vibrio cholerae*. The Instituto Oswaldo Cruz is also participating in PulseNet Latin America.

### Argentina -

Since 2004, Argentina has been participating as an active member of PulseNet Latin America. The standardized PFGE protocols for *Escherichia coli* O157, *Salmonella* spp. and *Shigella sonnei* were implemented at the National Reference Laboratories as well as a National Database for each pathogen. For *E. coli*, a total of 841 isolates have been analyzed which showed 400 unique *Xba*-PFGE patterns; for *Salmonella* spp., 550 isolates have been analyzed, with 227 unique *Xba*-PFGE patterns; and for *S. sonnei*, 174 isolates with 137 unique *Xba*-PFGE patterns. Among the isolates included in each database, were sporadic and outbreak associated strains from the period

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## Tour of the Network

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2004-2006, as well as isolates recovered previously that were analyzed in order to know the distribution of the different subtypes circulating in the country in the last few years.

Between 2004 and 2006, a total of 15 outbreaks caused by different *Salmonella* serovars were confirmed by PFGE and a cluster of *S. Typhi* related isolates were identified from the laboratory. Most of the *E. coli* O157 strains subtyped were associated with sporadic cases of bloody diarrhea or HUS; however, two outbreaks that occurred at kindergartens were confirmed and several clusters of genetically related isolates were identified.

The application of PFGE as a routine subtyping method has been extremely useful both for cluster detection and for outbreak confirmation providing an important tool for the establishment of prevention and control measures.

## A Case Study and Evaluation of PFGE Protocols for *Enterobacter Sakazakii*

The INEI-ANLIS "Carlos G. Malbrán," of Buenos Aires, presented the study "Subtyping of *Enterobacter sakazakii* by PFGE," at the 4th International Meeting of PulseNet Latin America. This microorganism is associated with serious childhood infections, especially in infants, such as meningitis and the necrotizing enterocolitis (EN). *E. sakazakii* infections have high mortality rates and the organism is very resistant to low humidity and high temperatures environments. Most infections have been described in outbreaks associated with infant formula milk.

The objective of the work was to evaluate two protocols for the subtyping of *E. sakazakii* which since 2004 has been included as a category A agent in the list of pathogens of risk of the World Health Organization. The two protocols analyzed were: A) the standardized protocol for *Shigella sonnei* from the PulseNet Network;

and B) the protocol developed in the laboratory of Dr. Matthew J. Arduino from CDC.

The researchers concluded that with both protocols it was possible to obtain high quality PFGE gels, with an adequate number of DNA bands. However, the protocol for *Shigella sonnei* presented the following advantages: it is faster (a single incubation in lysis buffer); it is more economical (it does not require lysozyme and the enzyme *Xba*I is more economical than *Spe*I); and it provided a better distribution of the DNA fragments in the gel, making analysis easier.

The *S. sonnei* PulseNet protocol was applied for the analysis of 21 isolates of *E. sakazakii* recovered in Argentina from different batches of a single brand of imported powdered infant formula. The *E. sakazakii* isolates studied were genetically diverse, see figure 1, suggesting that different sources could be implicated in the contamination of the infant formula.



### CONGRATULATIONS!

At the 2006 Annual PulseNet Latin America Meeting held in Buenos Aires, Dr. Bala Swaminathan was presented an award for his continued efforts toward building and improving the PulseNet Latin America network. DR. SWAMI, THANK YOU VERY MUCH!

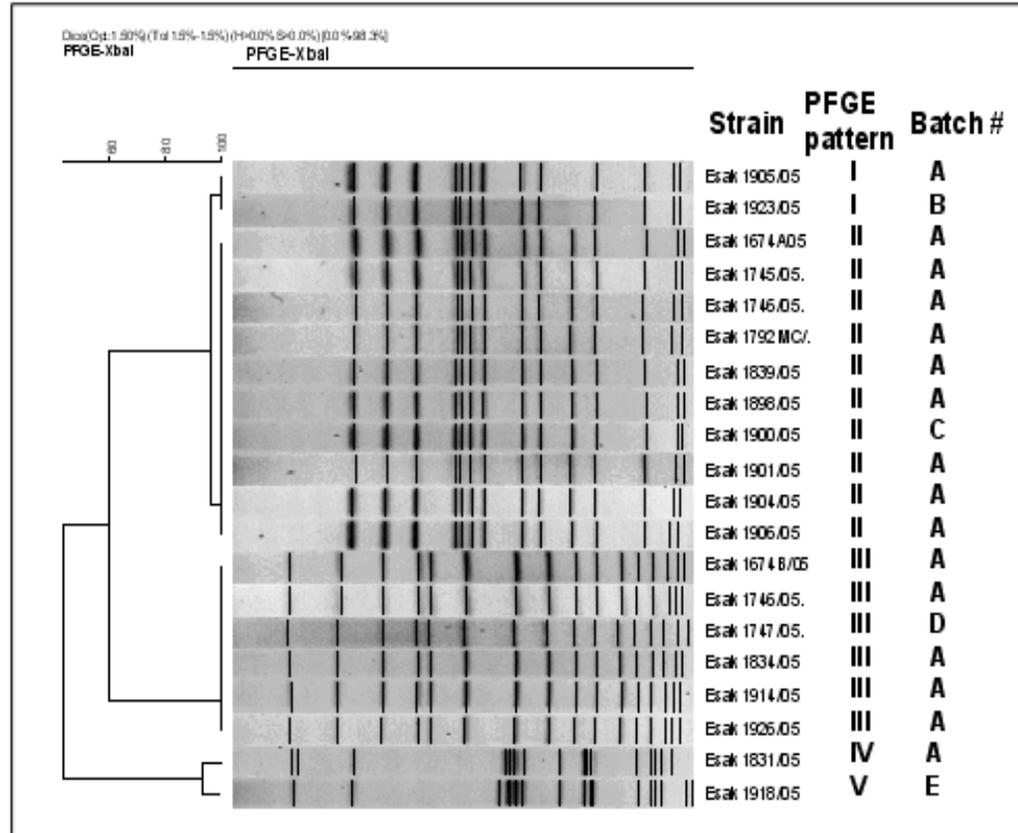


Figure 1 Genetic Diversity of *E. sakazakii* Isolates by PFGE-*Xba*I enzyme

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