PulseNet PFGE Protocol Development
A Historical Perspective

Mary Ann Fair
Division of Foodborne, Diarrheal and Mycotic Diseases
PulseNet Laboratory, CDC

SAFER • HEALTHIER • PEOPLE™

PAST

SAFER • HEALTHIER • PEOPLE™

PFGE - Major Developments

- In 1980s, several different electrophoresis techniques developed to separate >25 Kb DNA.
  - Can not be separated by conventional electrophoresis
- PFGE (Pulsed-field Gel Electrophoresis) - 1984
  - Schwartz and Cantor alternated electric field during electrophoresis to separate entire chromosome.
  - Molecules migrated forward in zig-zag fashion with some skewing of lanes.
- CHEF (Contour-clamped Homogeneous Electric Field) technology reported by Chu, et al - 1986
  - 24 electrodes in hexagonal shape

PFGE and Epidemiology

- Large outbreak of foodborne illness occurred in western US in late 1992 and early 1993.
- *E. coli* O157:H7 implicated in outbreak.
  - PFGE patterns of isolates from patients and hamburger patties were the same.
- Results of laboratory investigation published by TJ Barrett, et al in December 1994.
- PFGE data more epidemiologically relevant than Shiga-like toxin, plasmid and antimicrobial testing.
    - Able to group strains that were alike
    - Able to focus investigation

Need for Standardized PFGE Protocols

- CDC received many requests from state public health and other laboratories for PFGE testing.
  - CDC had limited staff and resources.
  - PFGE protocols were time and labor intensive.
    - 3-4 days to a week or more
    - Many different reagents, enzymes and electrophoresis conditions used
    - Inter-laboratory comparison of results was not possible
      - Not standardized

The First Standardized PFGE Protocol in 1996

- CDC developed "standard" protocol for PFGE subtyping of *E. coli* O157:H7.
  - Compared and combined methods from 5-6 different labs
  - PFGE results in 4 days after receipt of culture
  - Two PFGE workshops held at CDC in **1996**
  - Trained personnel from 17 different laboratories
  - Validation study to determine reliability and reproducibility of "standardized" PFGE protocol
    - 64 *E. coli* O157:H7 strains tested by 10 labs

First "Update" Meeting in 1997

- Meeting at CDC in **January 1997** concluded there was a need for "rapid" PFGE protocol.
- CDC began work on modifying protocol
  - Time reduced to 48 h for comparable results.
- In late 1997, Dr. Romesh Gautom from WA Health Department published in JCM **35**:2997-80.
  - Rapid pulsed-field gel electrophoresis protocol for *Escherichia coli* O157:H7 and other Gram-negative organisms in one day.

Standardized PulseNet PFGE Protocol in Late 1990s

- CDC and WA SHD collaborated in 1998 to combine the two PFGE protocols.
  - Changes in some reagents and conditions
  - Minor modifications in others
  - Protocol gave comparable results with the original 3-4 day protocol.
  - Reduced the amount of time for PFGE results
- 40 people were trained to do this “one day” PFGE protocol in 1998 and 1999 at CDC.

Major Changes

- SDS added to plug agarose
- Proteinase K (PrK) added to cell suspension
- Reduced amount of PrK added to lysis buffer
- Lysis incubation time reduced from overnight to 2 h
- Restriction digestion incubation time reduced
  - 4 – 16 h to 2 h
- SeaKem Gold Agarose used for electrophoresis gel
  - Run time reduced from 22 h to 16-19 h
  - Option to use 0.5% SKG agarose for PFGE plugs

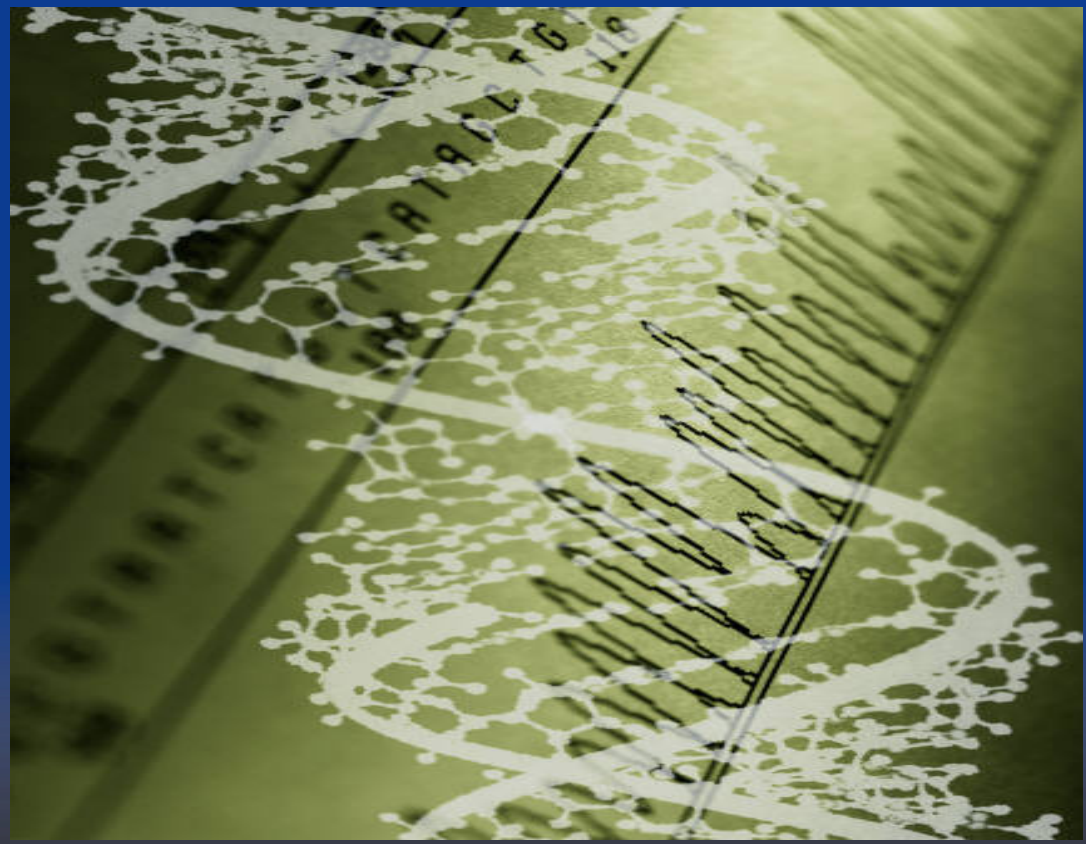
Effect of These Changes

- Simplified the number and type of reagents and chemicals needed for PFGE
- Served as a platform for development of standardized PFGE protocols for other organisms
  - Variations on a theme
  - Minimize inventory of reagents required
- Reduced the time required to obtain PFGE results from almost a week to two working days
- Allowed flexibility in planning work

Gel Agarose Used in Standardized Protocols

- 1996 CDC protocol used PFGE agarose from Boehringer Mannheim for gel electrophoresis
  - Product discontinued in early 1996 just after CDC's standardized protocol introduced
  - Substituted Pulsed-Field Certified from Bio-Rad
- 1998 "one-day" protocol used 1% SeaKem Gold (SKG) agarose
  - Higher strength and purity
  - Run time decreased
  - More expensive

PRESENT



SAFER • HEALTHIER • PEOPLE™

Cell Suspension Concentration

- Cell suspension concentration can be adjusted on:
  - Dade Microscan Turbidity Meter
  - bioMérieux Vitek Colorimeter
  - Spectrophotometer
- Values depend on type of tube used
- Each lab may have to determine value empirically
- Initially thought higher value was better, but paradigm is shifting to “less is better.”

Molecular Weight Size Standards for PFGE

- CDC used species-specific strains for size standards in reference lanes from 1996 until 2001
  - *E. coli* O157:H7 – G5244
  - *Salmonella* – *S.* ser Newport AM01144
  - *Shigella sonnei* – F2353
  - *Listeria monocytogenes* – H2446
  - *Clostridium perfringens* – CPERF1
- Lambda ladder was not used as MW marker for PulseNet
  - Poor resolution of higher bands
  - Width of bands is not uniform
  - Lot to lot variations

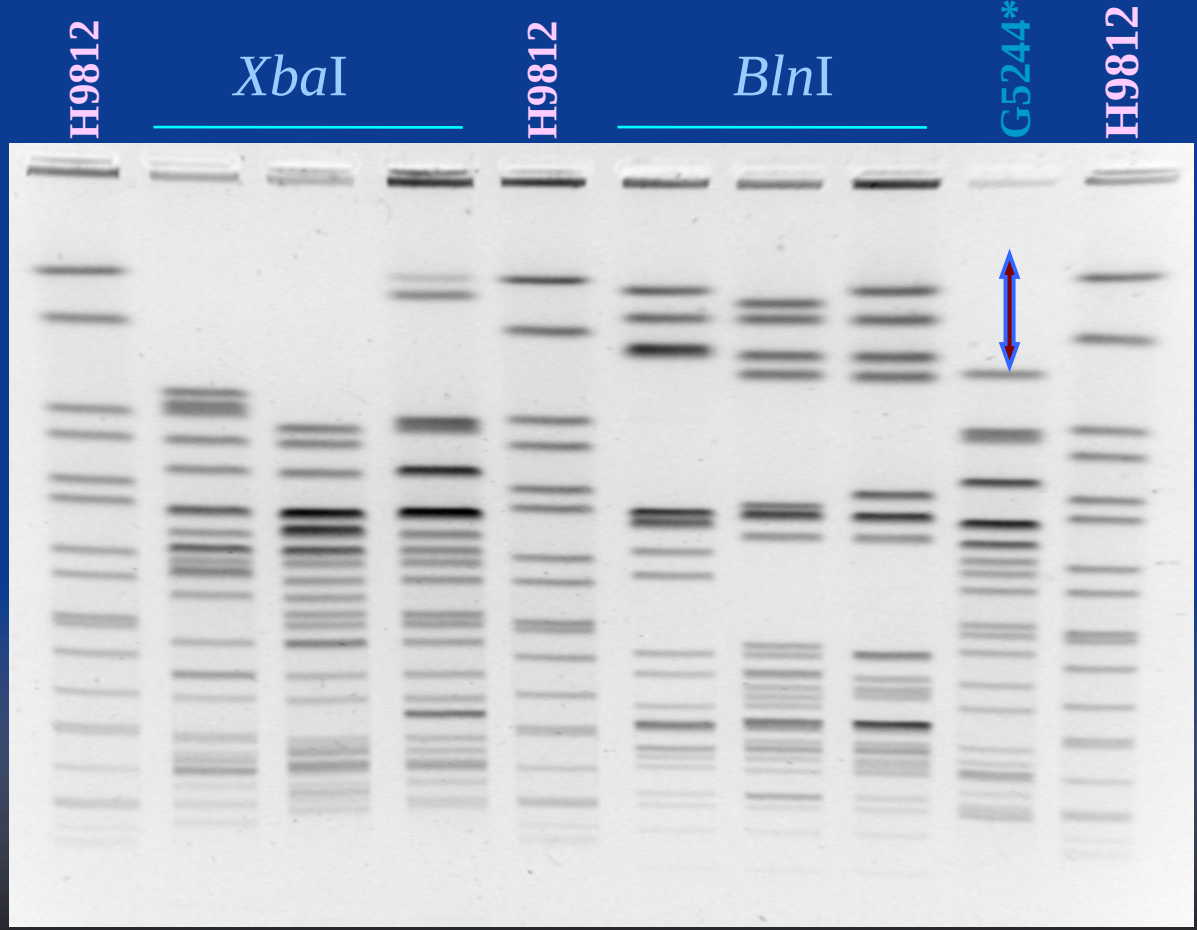
"Universal" Molecular Weight Size Standard

- CDC recognized the need for a "Universal" Size Standard.
  - Increasing number of organisms tracked by PulseNet.
  - PFGE plugs of several standards had to be made and tested.
  - Bands in some standards did not cover all PFGE patterns generated with second enzyme
    - *E. coli* O157:H7 and *Shigella* strains
- In late 2000, a gel image of a *Salmonella* ser. Braenderup strain restricted with *Xba*I was sent on routine gel from one of the participating PulseNet laboratories.
  - Had even distribution of bands

PulseNet Universal Standard Strain

- This *Salmonella* ser. Braenderup strain evaluated further at CDC
  - Band sizes ranged from 20.5 Kb – 1135 Kb when restricted with *Xba*I
  - Found to have a stable PFGE pattern after serial transfers
  - Sensitive to antibiotics used to treat *Salmonella* infections.
- Designated as H9812 and is the “Universal” standard or reference strain for all PulseNet test organisms.

Comparison of H9812 and G5244 on *E. coli* O157:H7 Gel



Standardized PulseNet PFGE Protocol Training

- Personnel from ~ 60 different US city, county and state health departments have been trained at CDC or US PulseNet Area labs.
- Personnel from ~20 different countries have been trained at CDC.
- CDC assisted with PFGE training for three PulseNet Asia Pacific and two Latin America workshops
- Consultation with PulseNet Europe

Advantages of Standardized PFGE Protocols

- Reproducibility of results
  - Intra- and inter-laboratory
- Allows real-time subtyping of pathogens for:
  - Enhanced cluster detection and public health response.
  - Prevention.
- Rapid exchange of accurate information and data between different laboratories.
- PFGE is still considered the gold standard for molecular subtyping.

Use of trade names and commercial sources is for identification only, and does not imply endorsement by CDC or U.S. Department of Health and Human Services.

Acknowledgments

Many CDC and Public Health Laboratory Colleagues

SAFER • HEALTHIER • PEOPLE™