

Summaries of Breakout Session 2
10th Annual PulseNet Update Meeting
April 3-6, 2006

New PulseNet Users

Facilitators: Kelley Hise (CDC) and Mary Ann Fair (CDC)

Note taker: Amy Camba (CDC)

1. **Turn around time for PFGE results**, especially as it applies to BT grant proposals:
 - a. 96 h upon receipt in the enteric/serotyping lab but varies per organism
 - i. This is time listed in the new BT grant proposal
 - ii. *E. coli* O157:H7 and *Listeria* should remain the priority organisms for “real-time” PFGE subtyping
 - b. *Salmonella*: is it a priority in labs?
 - i. Priority after *E. coli* O157:H7 and *Listeria*
 - ii. The labs should do the best they can with resources and personnel
 - iii. Serotypes associated with clusters or outbreaks should be given priority
 - c. Guidelines are vague as to when turn around time should start; labs don't know if they should start time from when the isolates are received in the enteric lab or in the PFGE lab
 - i. Because many labs do not have control over how long the isolates will be in the enteric/serotyping lab, they should start their time with receipt in the PFGE lab
 - ii. Improve communication between Enteric and PFGE laboratory to help minimize turn around time
2. **TIFF Size**
 - a. Gel resolution: appropriate TIFF size for submission is ~300-400 KB
 - b. Please refer to the “Image Acquisition with Gel Doc Systems” document (page 2) that was provided in the 2006 PulseNet Update Meeting Notebooks for directions on how to save TIFFs with the appropriate file size. Detailed instructions will be available in the latest version of the SOP for Image Acquisition. All SOPs can be found under the “QAQC Manual” conference on the WebBoard.
 - c. The above instructions only apply to TIFF images saved on Gel Doc XR (or higher) systems
3. **Unique Pattern List (UPL)**
 - a. Labs should maintain a local unique pattern list to see if patterns have been seen before in their databases
 - b. Instructions describing how to create a local unique pattern list are on the WebBoard under the “Important PulseNet Documents” conference
4. **WebBoard**
 - a. The “Important PulseNet Documents” conference is an useful place for participants to visit to find many different PulseNet documents
5. **BioNumerics v. 4.5**
 - a. This version of BioNumerics is not being used at CDC yet. Therefore, you may upgrade to this version if you have it; however, **CDC will not be able to troubleshoot any problems** you may encounter.
 - b. CDC will be upgrading to BioNumerics v4.5 sometime mid-year
6. **Certification/PT**
 - a. Priority in which organisms to get certified for:

- i. First get certified in the organism(s) that is highest priority in your lab. This may differ from lab to lab. Then get certified for (1) *E. coli* O157:H7, *Listeria* and then (2) *Salmonella*.
 - ii. May depend on priorities and available personnel in each participating lab
 - 7. Priority in which organisms to subtype**
 - a. *E. coli* O157:H7 and *Listeria* are top priority for real-time subtyping
 - i. If you cannot subtype these organisms in a timely manner (see above), please send your *E. coli* O157:H7 to your area lab and your *Listeria* to CDC (all *Listeria* isolates should be sent to CDC anyway—just make sure to indicate that PFGE is to be performed on the DASH form)
 - 8. Requests for PFGE lab and BioNumerics Training**
 - a. Shari Rolando from APHL asked for training requests in the APHL survey earlier this year
 - i. This list has been sent to CDC
 - b. If you have additional requests not captured by this survey, please send an email to pfge@cdc.gov with “Training Request” in the subject
 - i. Please indicate what kind of training you wish to receive (i.e. PFGE Lab, Beginning, Intermediate or Advanced BioNumerics training)
 - ii. If you are requesting BioNumerics training, please indicate some of the things you would like to learn in the class
 - 9. Sharing culture information and other data with the enteric labs in order to reduce amount of time spent on data entry**
 - a. Some participants are doing this through the sharing of Access databases
 - b. There was a PulseNet News article in the Fall 2004 issue (Volume 3, Issue4) regarding this; go to http://www.cdc.gov/pulsenet/pulsenet_news.htm for more information
 - 10. Sometimes PFGE subtyping can be done before serotyping**
 - a. This reduces the time it takes to get PFGE results
 - b. The downside is that you may end up subtyping isolates (serotypes) that are not “high priority”
 - 11. Process for requesting certification sets**
 - a. E-mail the PFGE inbox (pfge@cdc.gov) and request for certification sets
 - b. Please put “*organism* Certification” in the subject (ex: *Salmonella* Certification)
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QA/QC Program

Facilitators: Jennifer Kincaid (CDC) and Deb Sheehan (APHL)

Note taker: Robin Broeker (CDC)

- **Proficiency testing (PT)**
 - ◆ Random proficiency testing (PT) instead of the current set up where the states know when the samples are coming: People seemed to like this idea however; there is still some concern that random PT is not quality assurance/control. Implementation or target date is fall 2007.
- **Laboratory certification vs. individual certification**
 - ◆ For larger labs, it has been suggested that only the lab has to be certified by the CDC, not each individual within the lab. An already certified supervisor can certify the individual in his/her lab but a good in-house QA/QC program is needed. This shifts responsibility to the lab.

- ◆ The area labs decided that this was not necessary since certifications are being processed in a timely manner. May be something for the international networks to consider if interested.
- **Process of being certified**
 - ◆ Gel certification is required to run the gels in the lab. Certification is required for each individual organism.
 - ◆ Analysis certification is required to analyze the gels and upload the results to the national online database(s). Once certified, the person will receive a Key Fob.
 - ◆ The benefit of certification to the participating lab is to allow access to the database(s).
 - ◆ The gel/bundle file is reviewed upon receipt
 - ◆ Current turn around times is a few days.
- **Area labs**
 - ◆ Can provide training.
 - ◆ Can have area lab representative come to a participant's lab to help with set up and running the PFGE protocols.
- **Information on WebBoard**
 - ◆ Band marking tutorial
 - ◆ Laboratory Standard Operating Procedures (SOPs)
 - ◆ All important PulseNet documents
- **QC/QA Manuals**
 - ◆ CDC provided the QA/QC manual via WebBoard in 2005.
 - ◆ CDC's manual is to be used as a template or guideline for in-house programs.
 - ◆ Some states have own in-house QA/QC manual.
 - ◆ Virginia has a very thorough QA/QC program which, for example, includes things such as regular temperature checks. Refer to Denise Toney's presentation for more details on their program (http://www.aphl.org/conferences/pulsenet_update_meeting_2006/files/2006/Thursday/38%20Toney_PulseNet_QAQC.pdf).
- **TIFF image troubleshooting**
 - ◆ *BlnI* enzyme creating ghost bands: *BlnI* is temperature sensitive so suggestions included ways to minimize temperature fluctuations such as keeping the enzyme on ice.
 - ◆ Temperature of cooler water can affect TIFF image: Suggestions included recording temperature to see if there are fluctuations during the run. Also, using a NIST thermometer to make sure the reading of the cooling machine is correct.
 - ◆ For fuzzy bands, try cleaning the filter but also talk with BioRad.
- **Protocol for *Escherichia coli*, *Salmonella*, and *Shigella***
 - ◆ Question brought up about why there are three separate certification sets when the protocol is nearly identical. Three reasons given: (1) people have problems remembering to change the conditions (ramp times, temperature) to suit each organism; (2) *Shigella* analysis is hard to perform; (3) people new to PFGE may not recognize the differences.
- **Standard for certification, *S. ser. Braenderup*, H9812**
 - ◆ Question brought up about doublet in standard for certification being marked but not when analyzing gels otherwise. This doublet occurs at

approximately 180kb for *E. coli* and *Salmonella*. Reason being that some labs cannot always resolve the doublet during routine runs. For the future, CDC is looking at the option that the analysis certification may no longer include the marking of the H9812 patterns.

General Equipment Issues

Facilitators: Nancy Garrett (CDC), Ron Gladden and Scott (Bio-Rad)

Note Taker: Nehal Patel (CDC)

Topics Covered: Image acquisition, turbidity meters, PFGE equipment maintenance, review of different brands and models of PFGE equipment, and importance of proper image acquisition

Number of people attended: 25 total

Concerns and solutions expressed:

1. **New XR model** is less sensitive than GelDoc 1000, 2000

--BioRad suggested playing with the focus on the computer rather than the imager.

2. **Images are blurry.**

--BioRad suggested cleaning the filter. BioRad rep Scott showed the way to clean it on the Gel Doc XR machine that was present at the meeting. He mentioned that the filter can be cleaned by unscrewing the filter and washing it with warm water. Agarose tends to build up inside; also make sure to freeze the image and then turn off the UV light to minimize the build up of the agarose.

--Other suggestions:

- change LCD board –one lab person said this only made things better for a short time
- call the BioRad rep for your area and ask them to come out and look at the equipment
- if less intense, check and make sure all the bulbs are working; some may need to be replaced
- try to focus on the top wells of the gel and make sure the iris is completely open

3. **Curving of images** on both sides: Hammock effect

--try not to let gel sit too long after pouring the gel. Also, during imaging, try to zoom out a little bit more; this might help the problem when acquiring the image

4. **Calibrating turbidity meters?** How to order turbidity meter for the labs?

--Order information for the Dade Microscan Turbidity Meters was provided to individuals that needed it, including appropriate catalogue numbers since this item does not appear in the catalogue or on website. To order, contact Dade Behring at 1-800-242-3233 and ask for "inside sales." The turbidity meter is catalog number B1018-66 and should cost around \$910.24.

--Calibration suggestions were given by Efrain Ribot (CDC), who also suggested talking to MaryAnn Fair (CDC) as she is the person who takes care of this. (Maybe we can have MaryAnn give a short update on this topic next year at beginning of one of the breakout sessions). If want to contact Mary Ann, email her at MFair@cdc.gov.

--Some suggestions were to check the battery and maybe replace battery. Also make sure it is fully charged before using. Make sure to plug in and charge maybe once a week for a few hours—not sure if over charging is possible, but someone mentioned that overcharging may be an issue like with a cell phone.

New and Used Protocols

Facilitators: Kara Cooper (CDC), Eija Hyytia-Trees (CDC), and Kristy Kubota (CDC)

Note Taker: Michele Bird (CDC)

I. Future of MLVA

- Discussions from participants about suggested implementation: Initially all labs may continue to PFGE all isolates. In some limited cluster situations (known limited discrimination/diversity) additionally perform MLVA and compare results
- Quick tally: ~50% of groups have sequencers in their labs though not all groups routinely use.

II. MLVA Validation Overview

- 15 labs participating
- Phase I: CDC Sent isolates to labs for MLVA
- Phase II. Participating labs choose routine isolates for MLVA and PFGE subtyping and will send results to CDC for comparison/analysis.
- Currently, CDC is providing the primers (for validation process). However, in the future, CDC will provide the sequences to labs to make/purchase their own primers.

III. MLVA general issues:

- DNA in MLVA protocol at this time does not require purification. Crude DNA (boiled template-10mins) works well.
- Approximate cost of MLVA (i.e. *E .coli*) per isolate is about \$15. Might go down if number of target sites is reduced.
- Individual state and local health departments are encouraged to contact Beckman or ABI for specific sequence equipment troubleshooting concerns.
- Beckman and ABI are offering some equipment and or supplies at reduced costs.
- General concerns about reliability among users include:
 - # base repeats/base pair size differences
 - primer stability/quality
 - software conversions/field information consistency
 - capillary issues (shelf-life/storage life)
 - database memory \pm dose till capacity observed
- Database Memory: When memory close to fill capacity, system more likely to inappropriately identify fragment/allele size
- Instrument maintenance:
 - Capillary usage approximately 100 reactions. Capillary can stay in machine for routine use approximately 1month. If the capillary is not being used routinely, it is recommended that it be removed from the instrument and stored at 4°C.
 - Gel can be kept in instrument (regular use) for 10-14 days. After 14 days, it is recommended that the gel be discarded. Labs are advised not to mix gels from multiple uses/lots.

IV. **Sequence/Sequencer Issues:**

- Some laboratories have a sequencer but lack the appropriate software (specifically fragment analysis)
- Single capillary systems for MLVA are not recommended; 8-16 capillary instrument is necessary
- What is a lab with just a few *E. coli* a year to do? What is their role in the advent of MLVA for this typing scheme? Back and forth discussion of whether the lab should run sequencer for a few isolates or if the isolates should be sent to a lab better equipped for routine MLVA typing such as an area lab. Is it possible for these sequenced-based protocols to be modified for a "low volume" lab?

V. **PFGE Troubleshooting:**

- SDS or no SDS? CDC Experience indicates that the removal of SDS from the plug prep does not alter the expected results; however this process has not been officially validated. In the interim, participating labs are encouraged to establish what will work best in their lab and provide results that are reliable and consistent.
- Assure that the distilled water is of good quality, particularly when making up stock/house buffers

Salmonella Serotyping

Facilitator: Patti Fields (CDC)

Note taker: Susan Van Duyne (CDC)

I 4,[5],12:i:-

There was discussion about how people should report these isolates to PulseNet and to PHILS. Patti would like to have the isolates reported with the actual antigenic formula (4,12:i:-, 4,5,12:i:-, etc.). This applies to all other monophasic isolates as well. PulseNet wants the actual antigenic formula in the antigenic formula fields, but use the generic I 4,[5],12:i:- as the serotype.

Getting CDC antisera

Several people expressed frustration about getting antisera from CDC for *Salmonella* serotyping. Patti stated that the antisera are available although occasionally a particular lot is pulled for re-labeling. Labs need to contact Patti (404-639-1748; PFIELDS@cdc.gov) if they have difficulty in getting antisera.

Salmonella Group C2

Susan explained that we are seeing PFGE patterns that cross over between 5 pairs of Group C2 serotypes. She explained that this is not surprising because these serotypes differ only in the expression of O factor 6. Susan stated that studies are in progress to study this further, results of which will be presented at next year's PulseNet meeting. Serotypes involved are: Hadar/Istanbul, Muenchen/Virginia, Newport/Bardo, Litchfield/Pakistan, and Haardt/Blockley.

Managing PulseNet Data

Facilitators: Jana Lockett (CDC) and Molly Joyner (CDC)

Note taker: Desmond Jennings (CDC)

I) Discussion of Tracking Outbreaks

a) Ways of tracking clusters/outbreaks within states

- i) Add CDC outbreak codes to local BioNumerics or Access databases
- ii) Use internal outbreak codes
- iii) Use judgment when deciding when to post clusters to WebBoard
- iv) When a cluster is detected, check national database to see if other states have matches; then post to WebBoard if no one else has done so
- v) Supply epis with information about historical frequencies, temporal info of cluster patterns
- vi) Comparing cluster/outbreak patterns to national database
 - (1) Limit search as much as possible when querying *Salmonella* database
 - (2) Use 60-120 day search for other databases
- vii) Distinguishing outbreak isolates from sporadic isolates
 - (1) Note seasonal trends of patterns
 - (2) Use charts & statistics tool to visualize pattern trends
 - (3) Ages of cases may provide hints
- viii) For future discussion: CDC may need to produce document/SOP for determining who is responsible for keeping track of outbreaks (should the posting party be responsible for providing updates; should CDC be responsible once it becomes multi-state, etc)
 - (1) Labs are unsure of their role in following up with clusters i.e. when a cluster is posted, does every lab have to continue to search for matches for the next 60 days, or should that be the posting lab's responsibility?

II) Record keeping systems

- a) Keep track of outbreaks using excel sheets with local & national outbreak codes, pattern names, source info, and whether or not it has been followed up
- b) Epis should provide summaries & updates to WebBoard when outbreaks are closed.
- c) Using new WebBoard version (<http://forumx.forum.cdc.gov:8080/default.asp?boardid=pulsenet>), attach a personalized image or picture (click on Options, My profile, Add, Remove, or Change Image).
 - (i) When scrolling through conferences, look for your image to keep track of which bundles/postings you've seen already
 - (ii) Some labs keep a notebook that keeps track of the bundles they have downloaded from the WebBoard
 - (iii) Some labs print conferences from the WebBoard. A useful tool to have in SiteScape/future versions would be a tool that allows conferences to be printed so that information doesn't get cut off (without having to copy & paste the conference in Word)

III) Creating reports from BioNumerics

- a) Exporting comparison info from BN to Excel: File→Export Database Fields→use tab-delineated fields and right-click in text window and select all. Copy & paste into an excel sheet.
- b) A script to export/download outbreak codes would be useful to have in the future

IV) Assigning pattern names locally

- a) Can right-click at top of main BioNumerics screen and choose “Add new information field” (Can also do this by choosing “Add new information field” under database menu). Create a new field to store local pattern names in.
 - b) Create a local unique pattern list in Excel or other program that includes pattern name, date named, and gel & lane information for that pattern.
 - c) Name patterns in consecutive order
 - d) In order to name patterns correctly, gels must be analyzed correctly and bands must be properly marked
 - e) This information can be found under the “Important PulseNet Documents” conference on the WebBoard under the “BioNumerics Training and Tutorials” topic
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Interpretation of PulseNet Data

Facilitator: Peter Gerner-Smidt (CDC) and Efrain Ribot (CDC)

Note taker: Lewis Graves (CDC)

Peter Gerner-Smidt made a comment concerning the Foodborne Pathogen Disease Journal “PulseNet Issue.” Each public health laboratory will be provided with a copy of the issue.

Question: When is a 1-band difference considered a match?

Answer: For cluster and outbreak detection without supporting epi-evidence: 100% match. If you have both primary and secondary enzyme patterns and there is a 1-band difference in the second enzyme pattern, the isolates are probably different; however, depending on the epi data they might be considered the same. If you consider more than a 1-band difference you will have to include many profiles that are not related at all. Is this true for every organism? Yes, all organisms.

Question: Common patterns, what to do with a clonal group, e.g. *Salmonella*?

Answer: Depends on the question you want to answer. For cluster and outbreak detection, consider the history of the pattern in the local and the national databases, e.g. a cluster of a pattern that occurs regularly is only important if it is BIG; a cluster of a pattern that caused a big outbreak years ago may be significant even if it is small. PFGE may not be very efficient for some organisms, e.g. *Salmonella* Enteritidis or *S. Typhimurium* DT104.

Question: For *Salmonella*, if there is a 2-band difference are they related?

Answer: Depends on the question that you are asking; for clustering they are most likely not related, but if the difference is found among isolates within an outbreak defined by epi-info all patterns from patients known to be epi-related should be considered. In this case, remember you are considering defined separate patterns in an outbreak, not all patterns that differ by 2 bands. You have to explore the possibilities; this is true for 1 enzyme or 2 enzymes.

Question: When should the epis be alerted to investigate a cluster for *Salmonella*?

Answer: If the frequency of the pattern is significantly higher than the historical background in the database, then the epis should be alerted and the investigation should be considered. Never wait for second enzyme data if the first enzyme indicates a cluster

Question: For *Salmonella* are there guidelines for the usefulness of 2nd enzyme or when should a 2nd enzyme be used?

Answer: The second enzyme guidelines are posted on the WebBoard in the "Important PulseNet documents" conference.

Question: Should travel information be uploaded to the database?

Answer: Yes, travel information should be included if known for both domestic and international travel.

Question: In the case of *Campylobacter*, (it has a high mutation rate) would you expect 100% match?

Answer: *Campylobacter* is a very special case; we do not use the data for cluster detection. We use the data for confirmation of relationships and outbreaks that are detected in some other way. What way? Well, if people have been eating in the same place and all have gotten *Campylobacter* or if they have participated in the same bike race and come down with *Campylobacter* then that would be an example of some other way of detection.

Question: What is the answer to a situation where you have a very slight shift in a band on a gel that can be seen visually and you are concerned about a possible match or because of a FOIA?

Answer: We sometimes find many isolates that seem to match by 100%, but have slight shift, but they must be within that 1.5% tolerance in BioNumerics to be a match. In these situations, contact the database team at CDC and make a comment in the WebBoard posting. Ultimately it may be necessary to run the isolates in question on the same gel/by more enzymes/by other subtyping methods, e.g. MLVA.

Question: When can you call a group of isolates a clone in PFGE?

Answer: Depends on the question you want to answer: for cluster detection consider only indistinguishable patterns, for attribution of food sources look at natural branches in the dendrogram in which case you may consider a branch a clone..

Question: Real-time MLVA typing vs. PFGE for *E. coli*?

Answer: There are good candidate loci for *E. coli*, but they are not finalized to date. MLVA is not replacing PFGE at this point and time.

Question: Third enzyme for *Salmonella*?

Answer: There is a recommended third enzyme in most protocols, but they are rarely used. Steve Dietrich has experience with *SpeI* for some serotypes.

Question: Third enzyme for *Listeria*?

Answer: Considering *SmaI* as a candidate for third enzyme.

PulseNet Funding

Facilitator: Bala Swaminathan (CDC)

Note Taker: Shari Rolando (APHL)

Overview of Funding Situation

In 2004, CDC's ELC budget for food safety activities totaled \$8 million (\$2M from the Emerging Infections Office and \$6M from the Food Safety Office). This money covered ELC activities such as PulseNet, STEC testing, food testing projects, etc.

In 2005, NCID saw a decrease in the overall budget, and the ELC budget took a part of this hit. Thus, about half the states were short-funded to get through what was thought to be a one-time funding crunch.

In 2006, the Emerging Infections Office saw a decrease of \$2M and the Food Safety Office (FSO) was also decreased about another \$1.6M. Thus, FSO decreased ELC food safety grants by \$200,000 (\$6M to \$5.8M) for PulseNet, STEC, and other food safety activities at the state and local level. However, those states in the April ELC funding cycle were only funded for nine months, i.e. through December 31, 2006; in January 2007 they will be funded for 12 months.

In preparation for the above cuts, CDC worked to put PulseNet language into the BT grants as a way of off-setting the expected cuts. Several states that asked for this BT money were able to make up for some of their cuts to ELC.

PulseNet Central is in discussions with Debbie Deppe (CDC) and others involved with the ELC grants to set priorities for this year's food safety requests. PulseNet will be considered as the highest priority, but STEC activities are also very important.

The funds set aside to support Area Lab activities go through different channels than the ELC PulseNet funds and large cuts may be seen in these budgets.

The goal of PulseNet Central is to get as much money as possible to the state and local participants so that PulseNet work can continue.

Continuation Requests

The evaluation process will be designed to get the maximum utility out of the remaining available funds. Submitters need to be realistic in asking for funds based on their true needs.

Requests will be scrutinized more than in the past as the ELC group is asking project officers (such as PulseNet Central) to be critical of all requests. Thus, the numbers in your ELC reports will be compared to your actual submissions to the National Databases.

Keep PulseNet requests realistic and base your estimates on the numbers of patterns your lab has submitted in the past. If your estimates are based on higher numbers of pattern submissions, be able to justify this.

PulseNet Central estimates the cost of PFGE to be \$12-15 per isolate, up to \$20 per isolate if second enzymes are completed.

Make sure that your PulseNet activities are highlighted as a priority in your requests. You should list not only pattern submissions but epidemiology output as well (investigations completed, recalls issued, etc).

Research projects will not be given high priority for funding.

Questions

- 1. How can public health labs meet the 96-hour TAT for *Salmonella* subtyping, as stated in the BT guidance?**
 - Last year, *E. coli* O157:H7 and *Listeria* were considered to be important isolates in terms of BT capacity if performed in a timely fashion. *Salmonella* is not considered to be a BT priority and does not fall within the purview of the BT guidance.
 - Labs should do their *Salmonella* isolates as quickly as possible and can upload their patterns without the serotype if necessary as long as the serotype designation is added later.
 - The bigger question is how to prioritize *Salmonella* subtyping. CDC has identified funds for a research project to answer this question so that the most epidemiologically useful laboratory data will be generated by PulseNet.

- 2. At the LRN meeting, the topic of how to determine TAT was a gray area. Depending on laboratory structure, it can be difficult to determine when an isolate reaches the public health lab and that it needs to be sent for subtyping.**
 - Language on when to start calculating TAT for *E. coli* O157:H7 and *Listeria* isolates was not specified in the BT grant. Each public health laboratory operates differently and the PFGE staff interacts with the enterics lab and specimen processing stations differently. Thus each PulseNet lab will need to determine a fair and accurate timing system based on internal operations.

- 3. Is the 96-hour TAT requirement tied to funding levels?**
 - Yes, *E. coli* O157:H7 subtyping should be completed within 96 hours for 100% of the isolates received. This is also true for *Listeria*, but this should not be an issue due to the low volume of isolates received. *Salmonella* TAT is not evaluated in the BT grant.
 - The 96 hours counts business hours only – not weekends or holidays if your PFGE laboratory is not operating on those days.
 - Timing to the hour is not relevant for PulseNet work. If the new guidance implied that specimens should be tracked by the hour, then the language needs to be changed, perhaps to indicate “4 days” rather than “96 hours.”

- 4. Completing the lab work in timely fashion only matters if epidemiologists follow up on the results rapidly.**
 - Every day can make a difference when an outbreak has been detected.
 - The BT grant TAT targets do not apply to any *Salmonella* isolates, nor do they apply to broth cultures or stool samples. The targets apply to pure cultures of *E. coli* O157:H7 and *Listeria*.

- 5. *E. coli* O157:H7 isolates are often mis-classified by clinical laboratories. It would be a waste of resources to subtype these isolates prior to confirming their identity.**

- This problem may be due to the non-specific nature of the BD antisera and the fact that the SSI antisera are not designed for confirmatory testing.
 - PCR may be a good alternative to these antisera, although such protocols will not likely be run on a daily basis and TAT may still be affected.
 - The bigger issue here is how to educate the clinical laboratories in your jurisdictions so that they perform more accurate testing.
- 6. When positive STEC broths are submitted to the public health laboratory, recovery of organism is often as low as 50%.**
- The Connecticut laboratory is one lab that has consistently high recovery rates from STEC broths.
 - CDC will host a meeting with the commercial laboratories in early May at which CDC will propose that all positive STEC broths be plated to at least SMAC and resulting suspicious isolates be confirmed as O157:H7. If negative, these broths will still need to be submitted to the public health laboratory for non-O157 isolation. An MMWR will also be published on this issue.
 - Additionally, CDC and APHL created an algorithm for testing positive broths submitted by clinical and commercial laboratories. This algorithm will be distributed after the May meeting.
- 7. Will the new ELC grants be on a yearly cycle?**
- Those labs with an April start date may be short-funded so that all labs will get onto the same schedule with a January start date. Final decisions on this will come from Debbie Deppe.
- 8. Is *S. aureus* subtyping considered part of food safety testing or nosocomial outbreak investigations?**
- This testing will not be given high priority by the PulseNet project officers
- 9. What are some other sources of funding besides the ELC and BT grants?**
- If you are willing to combine research with your public health activities and can submit R01 grant proposals in cooperation with an academic institution, Swami has contacts with the enterics group at NIH and he is happy to assist you with this process.
 - The two top sources for funding outside of CDC would be NIAID/NIH and CREES/USDA.
- 10. Will ELC funding be approved to purchase MLVA equipment? Is this grant year too early to ask for equipment and supplies for MLVA capability?**
- Most public health laboratories already own a sequencer. The LRN provides funds for this purchase.
 - Since CDC wants to roll out the *E. coli* protocol to a limited number of labs this fall, it would not hurt to ask for funding for such capability at this time.
 - Only a few labs will be asked to perform MLVA on the initial roll out. After 1-2 years of data are collected and reviewed, the utility of MLVA as an adjunct to PFGE will be determined. Labs that find MLVA to be more useful or simpler to perform in terms of work flow may choose to switch at that time.
 - The purchase of fragment analysis software would be a good use of BT funds. Include in your justification wording that indicates that in the case of a BT event where time is of great concern, it will be useful to have laboratory methods in

place that have the potential to perform identification and subtyping at one time. Be sure to ask for equipment, software, personnel, and supplies.

- PFGE will not be phased out anytime soon, partially due to the need to continue to collaborate with international partners who are now coming on board with PFGE.

11. Our lab is interested in MLVA but we do not have any funds to participate in the studies. PulseNet is becoming less of a priority for some public health labs.

- PulseNet staff should interact with their epidemiologists to ensure that the data that is generated is utilized and seen as relevant to public health. This will help keep PulseNet as a priority activity in the eyes of the Health Department and thus of the laboratory.

12. Is the BT funding intended for surge testing or routine surveillance work?

- The rationale behind putting *E. coli* O157:H7 and *Listeria* subtyping on the BT grant is to improve the response time for these two highly pathogenic organisms and to assure the capacity of public health labs to meet surge testing needs when they arise.

13. Software purchases are often difficult at the state level. Even low cost items can be considered as capital purchases and maintenance contracts are almost impossible to purchase with state funds.

- Purchasing departments can be educated on the need for certain items.
- Be creative in writing software purchases into the equipment purchase.
- CLIA failure can be used as an incentive to keep instruments properly maintained.
- Vendors will usually work with you so that your purchase of software is included with the invoice for the equipment.
- Since it is more difficult to add software later, it may be best to purchase the highest level available with the original instrument purchase.

The Regulatory Perspective

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The FDA is divided into centers. The Office of Regulatory Affairs gets samples from food or contaminated products and those samples get sent to labs for PFGE. The TIFFs are sent to an FDA center for analysis and then uploaded to the National Databases. Communication is done on a lab to lab or an investigator to investigator level.

CVM is the smallest center in the FDA and receives isolates from NARMS retail meat.

Some state labs do communicate with FDA labs and do send samples (ex. – NY). FDA in CA has a partnership with the state, but does not have concrete communication when it comes to lab results.

VA works with FDA mainly for shellfish testing and are heavy participants in FERN. VA does food training that partners them with USDA and FDA. They do most of their own testing and get help when it's overwhelming.

FDA cannot always share information with labs during outbreaks, so they are working on a protocol stating under which circumstances to share isolates or samples.

FERN is managed by the USDA and FDA and does select agent training. FDA does FERN training and will have a real-time PCR training this year mostly for state labs. FDA is still looking for a place to host the course.

FDA has seven field labs set up to run PFGE and generate TIFFs to send to CFSAN (Christine Keys) for analysis and she will give feedback/trouble shooting to the lab and generate reports (works closely with epis) and contact PulseNet central.

USDA and FDA are uploading patterns as close to real time as possible.