

# Highlights of Breakout Session II

The PulseNuts Talk Back



# Topics for discussion during this session:

- QA/QC initiatives
- CLIA issues
- State specific questions
- Open discussion

# Spectrophotometer or Turbidity Meter QC and Tips

- Issues to consider:
  - Are absorbances checked regularly according to manufacturer
  - Are filters checked periodically (this can be done by using a standard filter)
  - Use standard solutions from manufacturer or a McFarland Standard
  - Must check and document regularly
  - Establish dedicated ranges based on performance measurements and what range constitutes a failure
  - LOTS of variation between units (particularly Turbidity Meters), may require vendor recalibration or self-recalibration
- Tip: If culture is too old, autolysis and smearing will occur
  - Listeria may pose a problem if older than 24 h at RT
  - Campylobacter and Neisseria have a tight timeframe

# Camera Issues and Tips

- Alpha imager
- Little to no QC of cameras being performed
- May want to consider cleaning filters (orange) to reduce fuzziness – moisture and residue buildup over time
- Faint bands may result from the bulbs not working properly or burning out over time
- Suggest the documentation of when to replace bulbs and use stickers with the date to remind or denote date of change
- Could use a fluorescent marker or standard to monitor fluorescent intensity over time
- Cleaning recommendations??
- Don't leave gel in Gel Doc overnight
- Protect EtBr from light for optimal results

# Flow Rate Issues

- Slanted lanes may indicate the need for adjustment
- Loose agarose can alter buffer flow rate and increase temperature due to poor circulation
- How to check?
  - Determine the amount of time required to circulate 1 L of water through the pump and adjust to a rate of 1L/min
  - Adjust pump speed to yield desired flow rate
- Helps to verify ability of multiple CHEF units to produce “identical” results
- Recommended frequency – 2 times per year but some labs perform monthly



# Pipette Calibration and QC

- WA sends out pipettes to be recalibrated using a 4 point scale
- SD has a pipette tracker
- How critical is it to have a 10 point check? Does it make a difference for large versus small volumes
- Dye based kit for QC and calibrations (Artel)
- Service contractor performs maintenance, calibrations and certifications
- QC checks ranged from 2/year to 1/year
- Important to assure that your level of calibration correlates with the need for your methodology



## Scales/Balances and QC

- Set of weights; once a month you have to check your weights
- According to CAP you should be checking the balance for accuracy every day (??)
- Calibrated weights (10g, 5g, 1g, 500mg)
- Annual PM on Balances
- Depends on what is defined in your QA plan – could range from monthly calibrations to yearly

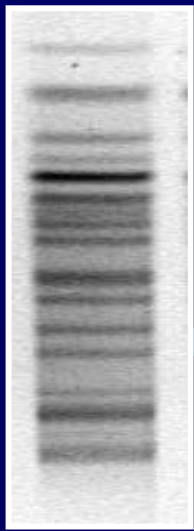


# Image Capturing QC and Tips:

- Saturated versus unsaturated – adjust iris and diaphragm
- Decrease band strength if exposed to UV too long
- Visualize manually, if good, then capture on camera
- Manual exposure (~1.0 sec)
- Freeze frame, “capture image”, turn off camera
- Adjust time (right intensity)
- Same image?
- If too long of an exposure, too little DNA then background will be increased

# Why are gel run times inconsistent?

- Electrode or building electrical issues
- Air bubbles
- Instrumentation leveling
- Chiller issues
- Room temperature issues
- Water quality and TBE buffer is crucial
- The use of same batches of TBE for running gel buffer and gel buffer will avoid minor differences in run conditions
- Differences in plug percentage – 1% agarose does not work as well as 1.2% (1% is specified in SOP???)
- Technique is subject to a wide range of variables that can effect this – qualitative not quantitative
- Size of the gel changes the conditions (10 well versus 15 well)
- Maybe CDC can work with BioRad to get loaner components when parts need repair



# How to address positive and negative controls?

- Global Standard serves this role, size analysis
- Digestion of controls verifies the enzyme works
- Electrophoresis of old versus new plugs to verify control plug preparation process
- Qualitative versus quantitative
- Can't have negative control for PFGE; more relates to PCR
- If things are not working, you will clearly see with PFGE
- Many of the general molecular guidelines do not specifically apply to the type of work performed in the PFGE laboratory
- Need to be careful in applying general molecular or PCR needs to testing such as PFGE; differences need to be clarified at a national level (role of CDC or APHL??)

# Other Questions and Answers

- Do you have procedures using calibration curves?
  - QC by instruments at Washington State, local service, make sure the local service is really doing the work they say they are
- How do you improve band quality?
  - Bln I – run small versus large gels
  - Better resolution on smaller gels
  - Zoom in will improve problems
- CAP – College of American Pathologists
  - Molecular biology
  - CAPs check list covers QC

# CLIA Goal

(from those who know):



- CLIA is looking for general good laboratory practices
- Consistency and completeness matters
- Document what you say you are going to do and have proof that you did what you said you were going to do?
- Document, review, document review.....;



# CLIA questions:

- Where are enzyme sheets and stored in freezer?
- Suggested keeping a paper for each lot
- Issue: should you keep enzymes in a box until you begin to use it??
- Are you allowed to keep in -20C without insert?
- Storage problems
- Where are your equipment maintenance logs?
- BioRad PM contract
- BioRad can QC machine in house – do you have the documentation?



## Is CLIA going to inspect PFGE?

- Grey area because we are not diagnostic and some are doing actual research
- If director decides this is an area requiring certification
- CDC might encourage labs to begin to be CLIA certified
- Cost of inspection might be prohibitive
- Dictated by laboratory director
- May be difficult to avoid if work and equipment is integrated with CLIA inspected areas; good justification for dedicated space

# CLIA questions:

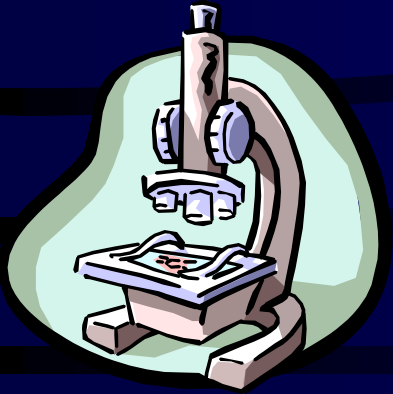
- What kind of PM do you perform on your gel box?
  - Company X not very helpful
  - DE gets the Vector Test done
- CDC has suggestions on how to check pump flow rate but labs have found these measurements inconsistent
- Check tubing for cracks, log temperature of chillers, create a check list
- BioRad Manual for PM was helpful
- Replace fuses and electrodes
- How do you document results
  - Data in computer
  - Date and gel number on picture in case they get separated
  - How long do you retain records





# Employee Competency Assessments

- Certification and Proficiency Testing
  - Internal and external
- Read procedures and related SOPs
- Comprehensive training protocol
- Direct observation
- Test competency samples and annual competency assessments
- Documented corrective actions and steps for retraining



# Issues for discussion

- CLIA and CAP overlap in a lot of areas
- Does anyone weight agarose before and after microwaving and then add water to make up the difference? YES
- Lots of variation when preparing TBE; need to document lot numbers; commercially purchased TBE tends to precipitate and there are slight changes in formulations
- How do we identify the changes in the new QA manual as compared to the older version?

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# Issues for Discussion

- Labs do not have the time to run all the statistical analysis that epis want and really don't have the understanding of it either
- What is CDC's recommendation for Band Widths?? Highlight lane just on the edge
  - some states have noticed that the lanes have been changed.
  - Will this be part of the script tool with respect to downloading of CDC changes/edits?

# Issues for Discussion

- Problem: Light Control Lanes
  - Solution: Increase cell suspensions
- Problem: New scripts application allowing us to see CDC's changes to our lanes takes TOO LONG to download
- BioRad should consider a trade in program so that laboratories could upgrade Gel Docs
- CDC should mandate all PulseNet labs have the same version of BioNumerics

# Comments

- Molecular Diagnostics – stands alone, no longer under bacteriology
- CLIA - Looking at new tests during inspections
- Standards versus controls
  - Suggest the use of high, med and low kilobase values from S. Braenderup as controls
  - Not quantifiable tests
- Standardization – the protocols standardization acts as a control for the comparisons between labs

# Summary and Comments

- NE region will be generating a complete list of issues of importance based on new CDC QA/QC manual to be e-mailed to everyone
  - Which items currently being done
  - The overall issue is that whether or not CLIA is regulating and inspecting the labs, labs are responsible for ensuring the overall quality of the results we are generating
- We want to be able to show proper documentation of lot numbers, temperature charts, pipette calibrations etc.

# Issues of Concern

- The difference between PT and competency and how it relates to our labs
- Regarding new shipping guidelines (IATA regs) does E. coli O157:H7 count as infectious or diagnostic??
- Regarding PT – if we are only getting annual PT isolates on those organisms we are analysis certified for, how do we demonstrate external competency?
- Can we get all the handouts for both epi and lab meeting posted to the webboard or distributed on a CD?
- Should we routinely be re-uploading NARMS isolates?

# Issues of Concern – cont.

- How often should we be able to hold regional meetings?
- Could regional meetings include
  - Group Mapper maintenance demos
  - Demo of changing electrodes
  - Demo of flow rate assessments
  - Overall use of Laura's Diversity Index (LDI), regional implications, second enzyme regionalness, etc



# Questions??

