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Life changes at SHL

- Iowa began sequencing in 2015
- PulseNet/Genome Trakr Lab
- 6 technologists currently perform sequencing across departments (Molecular/Virology, Microbiology(clinical), Research Division, and Environmental Microbiology
- Sequencing Projects
  - APHL TB sequencing
  - AMD Legionella
  - ARLN/HAI

Continued changes……

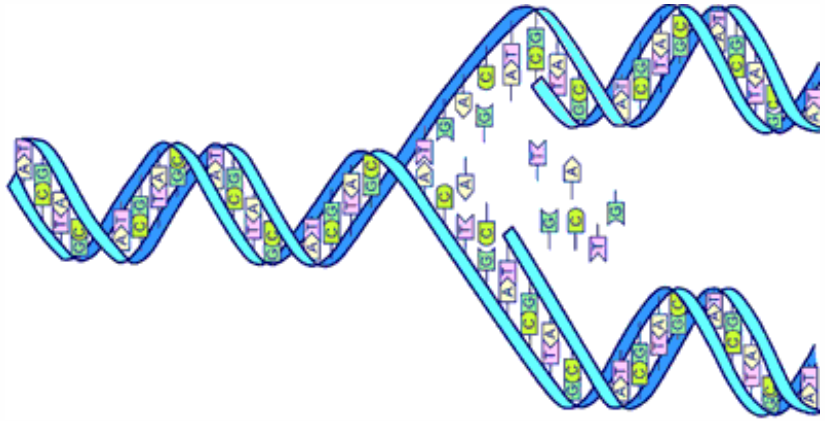
- CIDT
  - 2011-2014 1059 (average) isolates serotyped
  - 2015-2018 1447 (average) isolates serotyped
  - 37% increase in isolates for PulseNet
  - 2011 617 stool cultures, 171 pathogens isolated
  - 2018 1904 stool cultures, more than 850 pathogens isolated
- Workflow changes and validations have been ongoing to address this increase in workload

Current Workflow Status

- Enteric Bench
  - Culture
  - Conventional/~~Molecular~~ serotyping for Salmonella, Shigella, E.coli
  - Shiga toxin detection by Meridian EIA
  - Biochemical ID (TSI/LIA)
- PulseNet Bench
  - PFGE (E.coli/Salmonella)
  - WGS (E.coli/Salmonella/Listeria)

Future Workflow Status

- Enteric Bench
  - Culture
  - STEC PCR
  - MALDI ID (Salmonella)
  - Biochemical ID (TSI/LIA)
- PulseNet
  - Serotyping SeqSero and EC Typer
  - WGS (E.coli/Salmonella/Listeria)



Validation/Verification

- SHL Policy Statement
  - Activities needed for test method verification are a subset of those needed for validation. By completing a validation, it is required that the Verification requirements are also met.
  - Any major modifications, changes to the chemistry, new method variation, etc. of the procedure will require the test method to be re-validated. i.e. DNA Flex(yay!)
  - If a test method has been approved by outside agencies such as EPA or FDA, SHL shall follow the Verification step (7.2) … and meet the requirements specified in the method
  - If the test method has not been approved or if SHL deviates from the test method, then SHL shall follow the Validation step (7.1), followed by the Verification step (7.2).

Validation Requirements

- Identify the need
- Type of test method
- Applicability of test method
- Sensitivity
- Specificity
- Accuracy
- Precision and Repeatability
- Analytical Range
- LOD-Limit of Detection
- Nature of interferences
- Cost
- Reporting units
- QA/QC

Verification Requirements

- Prescribed agency guidelines (CDC methods)
- PT test results
- LOD
- Reporting Limit
- Sensitivity
- Specificity
- PPV
- NPV
- Accuracy
- Precision
- Holding time
- TAT
- Cost
- Demonstration of Competency

In the beginning…

- Initial Validation – based off of Minnesota's extensive Validation
  - 100 Salmonella isolates sequenced using CDC protocol
  - Sensitivity – (limit of detection not applicable) – sequencing coverage of 30X
  - Specificity – 10 E. coli and 10 Campylobacter
  - Accuracy – Existing serotype results (500), compare them to our sequences
  - Precision
    - within a run, between runs, with different operators, and with different instruments
  - Controls
    - Negative Extraction Control- Automated instrument
    - Negative control/Positive Library Control
    - PhiX Control
    - Index Controls

But wait! Why re-invent the wheel….

- Verification!
  - SHL decided on a verification after CDC's conference call with laboratory directors stating they would seek a waiver from the Center for Medicare/Medicaid Services.
  - This may change depending on how CMS reacts to the "black box" nature of the calculation engine at CDC.

Verification proposal

- 25-30 Salmonella isolates sequenced using CDC protocol
- Sensitivity – (limit of detection not applicable) – sequencing coverage 30X
- Specificity – 5 E. coli and 5 Campylobacter
- Accuracy – Existing serotype results (50-100) compare to sequences
- Precision
  - within a run, between runs, with different operators, and with different instruments
- Controls
  - Negative Extraction Control
  - Negative/Positive Library Control
  - PhiX Control
  - Index Controls

Competency Assessment

Annual Laboratory Competency Assessment for Microbiology, Section: PulseNet

Competencies: These 6 items MUST be included as part of the clinical competency assessment program.

1. Direct observation of routine patient test performance, including patient preparation, if applicable, specimen handling, processing and testing
2. Monitoring the recording and reporting of test results
3. Review of intermediate test results or worksheets, QC records, PT results, and preventive maintenance records;
4. Direct observation of performance of instrument maintenance and function checks
5. Assessment of test performance through testing previously analyzed specimens, internal blind testing samples or external PT samples
6. Assessment of problem solving skills

Assessment Result Key:
S – Satisfactory
NI – Needs Improvement
NA – Not Applicable

Name of Employee:	**Initial Assessment** □ **6 month Assessment** □ **Annual** □		
SHL Section: Microbiology			
Microbiology section: PulseNet/WGS			
Competencies:	**Date**	**Observer Initials**	**Assessment Result & Comments (if applicable)**
Has read and understands WGS SOP		n/a	
Demonstrates knowledge of ordering supplies			
Demonstrates use of appropriate PPE and safety practices			
Demonstrates knowledge of appropriate collection and shipping methods			

Direct observation

1. Direct observation of routine test performance, etc			
Performs proper sample quick entry in OpenELIS			
Demonstrates knowledge of rejection and acceptance criteria and related documentation (resolution form and adding QA events)			
Performs proper handling, labeling, preparation, and storage of samples as specified in SOP			
Describes how to prioritize testing			
Demonstrates capability of perfomance of Plug preparation			
Demonstrates capability in performing enzyme digestion			
Demonstrates capability in performing pouring of gel			
Demonstrates capability in running electrophoresis unit(DRIII)			
Demonstrates capability in documenting and analyzing gel			

Monitoring the recording and reporting of test results

2. Monitoring the recording and reporting of test results			
Knows how to build and print worksheets and reviews TRF to ensure correct test was ordered			
Records, scans, and reports test results satisfactorily (worksheets)			
Demonstrates ability in uploading gel information to CDC			
Demonstrates knowledge of reporting information to IDPH			
Demonstrates how to complete and release results in OpenELIS			

QC/QA review

3. Review of QC (Quality Control) records, PT results, and preventive maintenance records			
Understands how to load QC when building worksheet and edit QC in OpenELIS			
Correctly interprets (QC) and records QC on proper worksheet			
Knows how to process PT samples are they are received (sample accessioning, sample preparation, etc.)			
Participates in Profiency Testing (PT) and reviews results			
Knows location of instrument maintenance log book			
Capable of using the SHL intranet assessment tool to document corrective actions taken for lab PT's, client complaints, etc.			

Direct observation of instrument maintenance and function checks

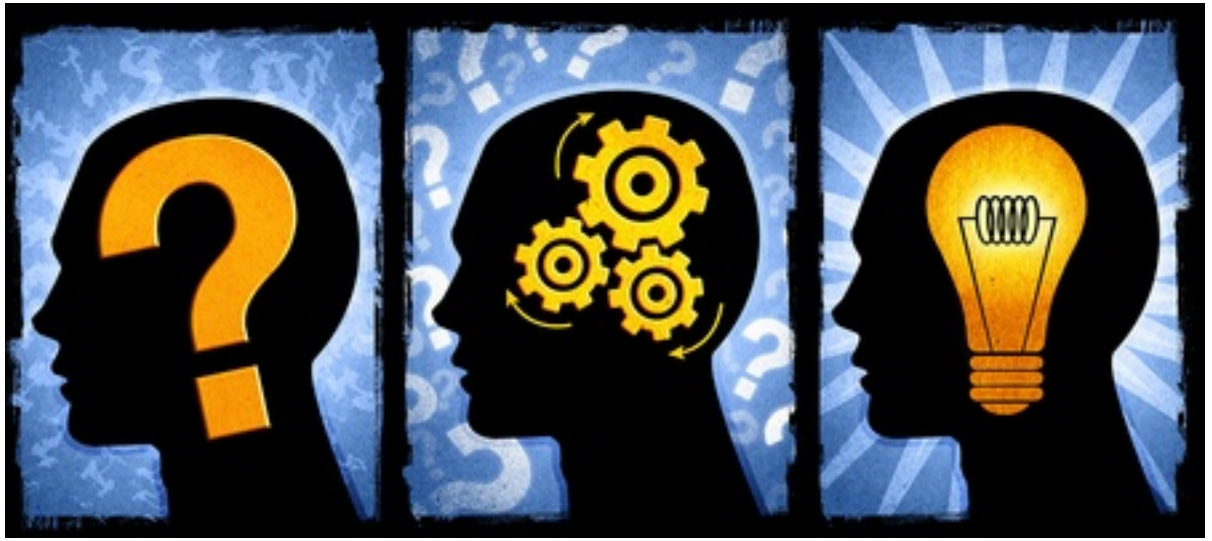
4. Direct observation of performance of instrument maintenance and function checks			
Properly cleans Buffer boxes and drains buffer when needed.			
Bleach and check flow every 6 months for each Buffer box.			
Demonstrates skills required for trouble shooting and checks equipment when used (water baths, gel doc) and takes corrective action when necessary			

Assessment through previously tested samples, internally blinded samples or external PT samples

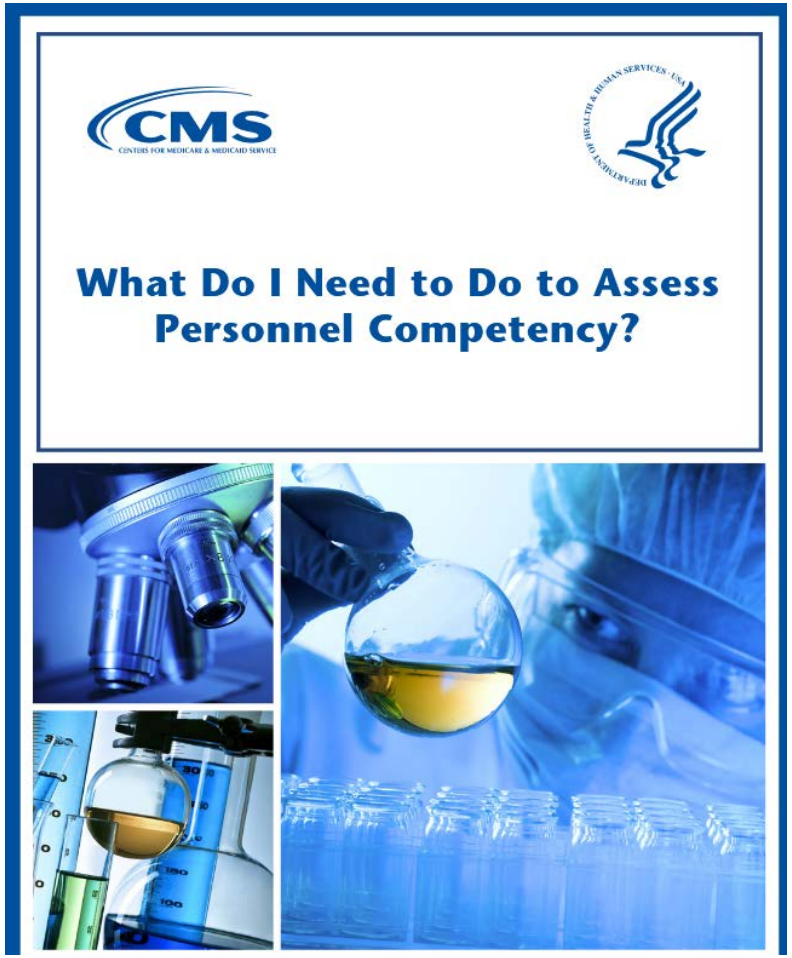
5. Assessment of test performance through testing previously analyzed specimens, internal blind testing samples, or external PT samples			
Demonstrates capability in analyzing gels			Worksheet number:
Demonstrates capability in reporting pattern names to IDPH			Worksheet number:
Demonstrates capability in reporting nosocomial results to clients			Worksheet number:
Demonstrates capability in discovering outbreak clusters			Worksheet number:
Demonstrates capability in reporting on Sharepoint			Worksheet number:
Demonstrates capability in relaying demographic information to CDC			Worksheet number:

Problem solving assessment

6. Assessment of problem solving skills			
Demonstrates Critical Thinking/Problem solving skills			See M:\Bacti\Competency\ Assessment of Staff Problem Solving



https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CLIA_Brochures.html



Individualized Quality Control Plan "IQCP"

- Do I have to use IQCP approach?
  - IQCP is "voluntary". However, if you do not participate in IQCP, your laboratory must perform 2 levels of external controls on each test system for each day of testing and also follow all specialty/subspecialty requirements in the CLIA regulations for **nonwaived** tests.
- PFGE was never inspected by CLIA at SHL, because SHL did not report PulseNet patterns to clinical partners.
- What happens now that serotyping is dependent on PulseNet?
- Can these "controls" be temporary? Can an IQCP remove the need for controls?
- https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CLIA_Brochures.html

THANK YOU
QUESTIONS?

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