Nebraska Perspective for Conducting PulseNet WGS Surveillance
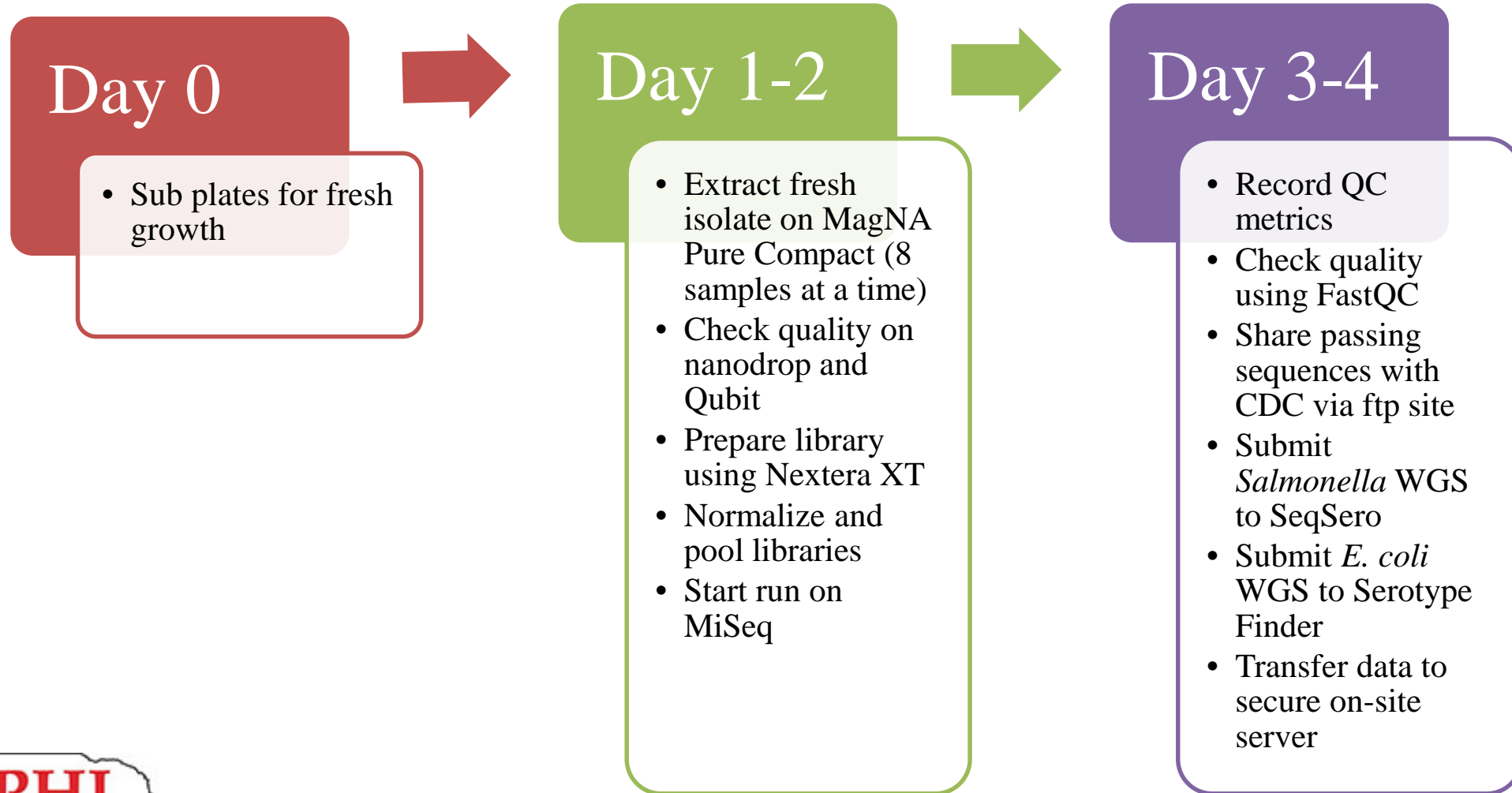
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Overview

- Current workflow

- Validation process

- Integration with PFGE

- Reporting

- Communication with Epis

- Future plans

WGS Workflow

Day 0
- Sub plates for fresh growth

Day 1-2
- Extract fresh isolate on MagNA Pure Compact (8 samples at a time)
- Check quality on nanodrop and Qubit
- Prepare library using Nextera XT
- Normalize and pool libraries
- Start run on MiSeq

Day 3-4
- Record QC metrics
- Check quality using FastQC
- Share passing sequences with CDC via ftp site
- Submit *Salmonella* WGS to SeqSero
- Submit *E. coli* WGS to Serotype Finder
- Transfer data to secure on-site server

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Turnaround Times

Run 1-2 times a week during Summer

Slower season (winter) fill runs with other samples
- *Yersinia enterocolitica* research project
- *Mycobacterium* research project
- *Francisella* research project
- EPEC research project
- Highly antibiotic resistant organisms
- Future CSF project
- Historical samples of interest

MagNA Pure Compact Validation

612 isolates were extracted on MagNA Pure Compact

Water samples were extracted alongside isolates as contamination control

All 8 lanes of Compact were tested in multiple runs

All lanes tested negative for DNA via Qubit

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WGS Validation

Ran some 2017 and all 2018 *Salmonella* and STEC isolate on

PFGE and WGS

- 449 *Salmonella* isolates

- 158 STEC isolates

Ran 5 previous isolates of *Listeria* on PFGE and WGS

Serotyping Validation

Salmonella fastq files submitted to SeqSero https://cge.cbs.dtu.dk/services/SeqSero/ or http://www.denglab.info/SeqSero	STEC fastq files submitted to SPAdes for contigs in fasta format (https://cge.cbs.dtu.dk/services/SPAdes/) STEC fasta contigs submitted to SerotypeFinder https://cge.cbs.dtu.dk/services/SerotypeFinder/
Listeria fastq files submitted to MyKmerFinder https://cge.cbs.dtu.dk/services/MyKmerFinder/	

Serotyping Validation

428 of 432 *Salmonella* isolates matched via PFGE and WGS (99.1% accuracy)

WGS	PFGE
Braenderup	Montevideo
Hindmarsh or Bovismobrifican	Infantis
Paratyphi B	Newport
4,[5],12:i:=	Heidelberg

141 of 141 STEC isolates matched via PFGE and WGS (100% accuracy)

5 of 5 *Listeria* isolates matched via PFGE and WGS (100% accuracy)

34 strains: WGS was able to type that were untypable by PFGE

Integration with PFGE

Currently PFGE and WGS done on every *Salmonella* and STEC isolate

WGS is validated

Plan to stop PFGE on March 15th

Start reporting *Salmonella* and STEC isolates based on WGS on March 18th

Reporting

WGS report will look similar to current PFGE report for the State

PFGE Report

Salmonella serotype Typhimurium
CDC PFGE Pattern: XbaI JPXX01.3123
Other Isolate ID: NPHL 190046
CDC Outbreak Code: None
PFGE analysis performed at NPHL for epidemiological purposes only.

WGS Sample Report

Salmonella serotype Typhimurium <>
Allele Code: SE1.0:5.1.2.3.1
Other Isolate ID: NPHLTEST2
Outbreak Code: None
Testing performed by whole genome sequencing.

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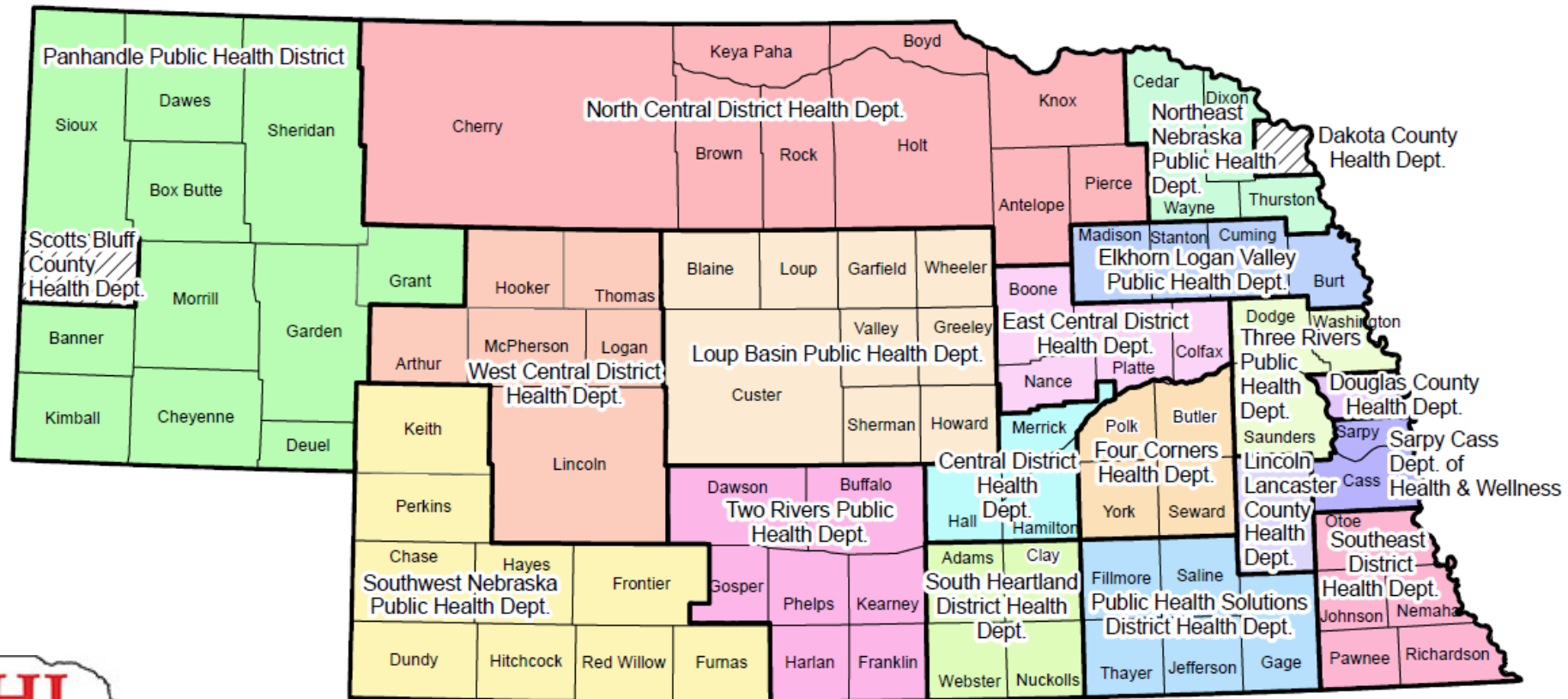
Epi Communication

State-wide partnership call with Epi's to prepare for switch

Explained how the reports will look similar

Explained turn-around-time changes

Set up bi-weekly calls with Epi's to keep communication open

Will work with Epi to determine cluster detection and outbreak investigation

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Current WGS Status

Sequence all *Salmonella,* STEC, and *Listeria* isolates (average year ~400 *Salmonella,* ~100 STEC, and <5 *Listeria*)

4 personnel trained and certified in WGS fastq file generation (library prep)

3 personnel certified in WGS analysis

1 person submitting WGS analysis certification within the month

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Future

Discontinue PFGE for routine *Salmonella* and *E. coli*

Purchase another MiSeq as funding permits

Begin using BioNumerics to analyze WGS data

Validate MagNA Pure 24 for automated extractions

Explore Nextera DNA Flex library prep kit option

Explore using MiSeq 300 cycle kit during slower times

Determine what constitutes a cluster for outbreak purposes

Report AST for *Salmonella*