Facilitating the implementation of NGS-based Diagnostic Testing in Infectious Disease Laboratories

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CDC’s QMS Risk-Based Approach to Next Generation Sequencing

- CAP Guidelines
- FDA Regulations
- CLIA Regulations
- ISO Standards

Quality Management System – provides the foundation to build upon
Challenges of NGS to Regulatory Compliance and Patient Safety on a PHL CLIA Certificate

• Use of non-validated, uncontrolled technologies.
• Experts in use and development of NGS technologies often less versed in clinical laboratory standards or regulations.
• Ever expanding laboratory activities can potentially impact patient care (and directly impact CLIA certification):
  • Patient identifiers de-coded offsite
  • Outbreak investigations and “research use only” testing
  • “Behind the scenes” testing
• Challenges compounded by complexity of novel technology and difficulty in interpreting specific CLIA regulations.
Diagnostic NGS at CDC Infectious Disease Laboratories

• Two diagnostic tests using NGS on the Roybal campus CLIA menu:
  • FVIII Gene Sequencing
  • Enteric Bacterial Identification

• Other NGS activities (unable to report at a patient level):
  • Pathogen characterization*
  • Phylogenetic analysis
  • Hospital infection control
  • Antimicrobial resistance/susceptibility*
  • Metagenomics/pathogen discovery*
    *in pipeline towards CLIA activity

• CDC reference labs are often “end of the line” for diagnostic testing: arguable need to provide this specialized testing to PHL partners and US population.
OID/CDC Efforts to Support Diagnostic NGS Implementation

• Challenge: Multiple, specialized laboratories. Re-inventing the wheel is impractical.
  • Solution: Generate ready-to-implement SOPs and forms, each made flexible for customization to individual laboratory needs.

• Resources available: Scientific, technologic, quality systems and bioinformatic expertise throughout organization.
  • Engagement: Provide a venue to communicate and define best practices.

• Desire to work with external partners.
The NGS Quality Workgroup meets monthly to identify challenges and gaps in laboratories performing NGS for both research and diagnostics.

- Lead: Rebecca Hutchins, started in 2015.
- Participation from multiple Centers: NCEZID, NCIRD, NCHHSTP, CSELS, NCEH.

The workgroup develops SOPs, forms, guidance, and tools to address the gaps.

Key success factors:

- Inclusion of laboratorians, bioinformaticians, and quality managers (NGS users).
- Interactive and inclusive discussions.
- Systematic approach.
- Surveyed NGS users to determine areas of greatest need from their perspective.

Hutchins, R. Manuscript in preparation
Alignment to Quality System 12 Essential Elements

• Workgroup output aligned to the 12 QSEs (Clinical and Laboratory Standards Institute).

• In 2015, a survey to NGS-using laboratories, identified the QSEs of Equipment, Personnel and Process Management to have the largest gaps and posed the greatest risk.

• These were prioritized to address.

Hutchins, R. Manuscript in preparation
CDC NGS Quality Workgroup: Output

- The Workgroup collaborated to develop guidance, SOPs and Forms for QSE’s Equipment, Personnel and Process Management.
- A total of 39 documents have been reviewed for external release.
- Multiple additional documents that have not been reviewed for external release (e.g. analytical SOPs) or are in “working drafts”.

Hutchins, R. Manuscript in preparation
These relatively simple forms are ready to implement and customizable. Such forms can save laboratories the work of creating *de novo*.
This procedure and form has been adapted by non-NGS laboratories, highlighting the strength of the quality systems approach to identify needs.

Hutchins, R. Manuscript in preparation
Process Control for the Wet and Dry NGS laboratories

Wet Laboratory

- Nucleic Acid Extraction
- QC Checkpoint
- Fragmentation and Size Selection
- QC Checkpoint
- cDNA synthesis (RNA only)
- QC Checkpoint
- Library Preparation
- QC Checkpoint

External Positive and Negative Controls

Dry Laboratory

- NGS Output
- QC Checkpoint
- Pre-processing Read trimming, Filtering
- QC Checkpoint
- Assembly
- QC Checkpoint
- Analysis

External Positive and Negative Controls

Hutchins, R. Manuscript in preparation
### Example QC Checkpoint Checklist

<table>
<thead>
<tr>
<th>QC Checkpoint (Process Step)</th>
<th>Method (SOPs)</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cDNA Synthesis</strong> (2.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Quantitate purity and concentration</td>
<td>Purity (choose one):</td>
<td><em>Purity: A$<em>{260}$/A$</em>{280}$ = 1.8-2.0</em></td>
</tr>
<tr>
<td></td>
<td>NanoDrop Nucleic Acid Quantitation Assay</td>
<td>Concentration: &gt; 500 ng in a 20-100 µL sample</td>
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<tr>
<td></td>
<td>Other ________</td>
<td></td>
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<tr>
<td></td>
<td>A$_{260}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration (choose one):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Qubit dsDNA or RNA Quantitation Assay</td>
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<tr>
<td></td>
<td>Quant-iT Assay</td>
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<td></td>
<td>Other ________</td>
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<tr>
<td></td>
<td>A$_{260}$</td>
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<tr>
<td></td>
<td><strong>Electrophoresis Instrument for NGS (choose one):</strong></td>
<td></td>
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<tr>
<td></td>
<td>TapeStation Assay</td>
<td></td>
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<tr>
<td></td>
<td>Bioanalyzer Assay</td>
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<td></td>
<td>BluePippin DNA Size Selection Assay</td>
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<tr>
<td></td>
<td>Other ________</td>
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</tbody>
</table>

*for RNA sample only*

<table>
<thead>
<tr>
<th>Library Preparation (2.8)</th>
<th>Concentration (choose one):</th>
<th>Concentration: &gt; 1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Quantitate concentration and confirm size selection</td>
<td>Qubit dsDNA or RNA Quantitation Assay</td>
<td>Electropherogram results: Single peak of desired size with no tailing and excessive broadening per lab specifications</td>
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<tr>
<td></td>
<td>Quant-iT Assay</td>
<td></td>
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<tr>
<td></td>
<td>KAPA qPCR</td>
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<td></td>
<td>Other ________</td>
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<tr>
<td></td>
<td>A$_{260}$</td>
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<tr>
<td></td>
<td>Concentration (choose one):</td>
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*The expected results included are based on standard NGS methods in use at the time of document development. The advancement of new methods and technologies may allow for successful sequencing with QC results differing from those listed in this document.*
Ad hoc Discussions to Determine Best Practices

• Consideration of external sequence data as a clinical sample.
  • Acceptance criteria driven by meeting defined QC checkpoints.

• Internal/external controls and individualized quality control plans (ongoing).

• Venue to communicate reagent recalls and identify reagent quality issues.
Work Group Expertise Provided Input to the CLIAC Federal Advisory Committee, April 2018

- Provided the public health voice at this session.
- Identified specific CLIA regulatory challenges and described CDC best practices to address:
  - Personnel.
  - Process control, including distributive testing.
  - System validation and re-validation.
  - Analysis (including record retention) and reporting.
- CLIAC recommended formation an NGS workgroup.

Hutchins, R: “Diagnostic NGS Challenges: CDC PHL Perspective”
CDC NGS Quality Workgroup: Future Direction

• The Workgroup is tackling the QSEs of Process Management, Organization, Information Management and Assessments:
  • NGS Method Validation: Guidance, Procedures and forms.
  • Individualized Quality Control Plan.
  • Quality Assurance planning.
  • Information Management Guidance (data file retention).
  • Proficiency testing
Next Steps

• Manuscript in preparation (including 39 documents and forms) on personnel, equipment and process control.
  • Will be publically available, but a “snapshot” as field rapidly evolves.

• Plan to strengthen collaboration with CDC’s Division of Laboratory Systems (CSELS) and APHL
  • Engagement and interaction.
  • Development of resources to support public health quality management of NGS-based testing.
Acknowledgements

• CDC NGS Quality Workgroup Members
• Office of Infectious Diseases/Office of the Director:
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• Division of Laboratory Systems/Center for Surveillance, Epidemiology, and Laboratory Services:
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  • Adeeba Saboor

Contact: vkd6@cdc.gov

Disclaimer: The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of Centers for Disease Control and Prevention.
CDC NGS Quality Workgroup: Approach

- Systematic approach to improve quality management systems for labs that perform NGS testing

**Identify Critical Risk Areas**
- Survey NGS labs
- Form risk mitigation strategies
- Develop QMS implementation plan

**Develop Quality Documentation**
- Develop consensus on best practices
- Develop SOPs and Forms to assure quality
- Publish documents for NGS lab use

**Evaluate for Effectiveness**
- Review and revise documentation as needed
- Engage with core groups to understand ongoing needs
- Address challenges via NGS workgroup input