

Diagnostic Next Generation Sequencing Challenges: CDC Public Health Laboratory Perspective

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CDC Public Health Laboratory Stakeholders

- CDC NGS Quality Workgroup: Comprised of **NGS users and quality managers** from across CDC, the purpose of this collaborative workgroup is to harmonize quality standards for NGS across CDC and provide laboratories with confidence in reported results.
- CDC NGS Quality Review Board: Facilitates **communication and coordination** between the Office of Advanced Molecular Detection (OAMD), Office of the Associate Director for Laboratory Safety and Science (OADLSS), OCCP, Center for Surveillance, Epidemiology, and Laboratory Services (CSELS), and the Centers, Divisions, and Branches which house NGS laboratories.
- Office of Infectious Diseases CLIA Compliance Program (OCCP): CLIA Laboratory Director Atis Muehlenbachs and the OCCP team work to **ensure patient health and safety** by maintaining and continuously improving the quality of clinical testing in CDC Roybal campus laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA).
- Collaboration with PHL Partners: Individual public health laboratories and APHL.

CDC Public Health Laboratory uses for NGS

Currently Operating under CLIA

- FVIII Gene Sequencing
- Enteric Bacterial Identification

Other Activities

- Pathogen identification and/or characterization*
- Phylogenetic analysis
- Hospital infection control
- Antimicrobial resistance/susceptibility*
- Metagenomics/pathogen discovery*
- Newborn screening
- Database generation & curation

* In pipeline towards CLIA activity at CDC

Challenges and the Efforts to Address Challenges-I

Personnel Challenges

- Training – NGS workflows are lengthy and diverse skill sets are required at different points in the workflow.
- Personnel performing post-analytical activities (e.g., flagging of problematic or unusual specimens for additional analysis) are not legally required to be trained or assessed for competency.
- Personnel with skill sets in computer science and bioinformatics are needed, however their training may not align with traditionally accepted degrees per CLIA.

CDC Public Health Laboratory Practices

- Training – Developed training SOPs to define the specific knowledge and skills necessary to perform different aspects of the NGS workflow.
- Competency – Developed processes to assess competency of personnel at all stages of the NGS Workflow.

Continuing Challenges: Difficult to qualify CLIA testing and supervisory personnel based on fit between their education and skills and the tasks completed in the NGS laboratory workflow

Challenges and the Efforts to Address Challenges-II

Process Control Challenges

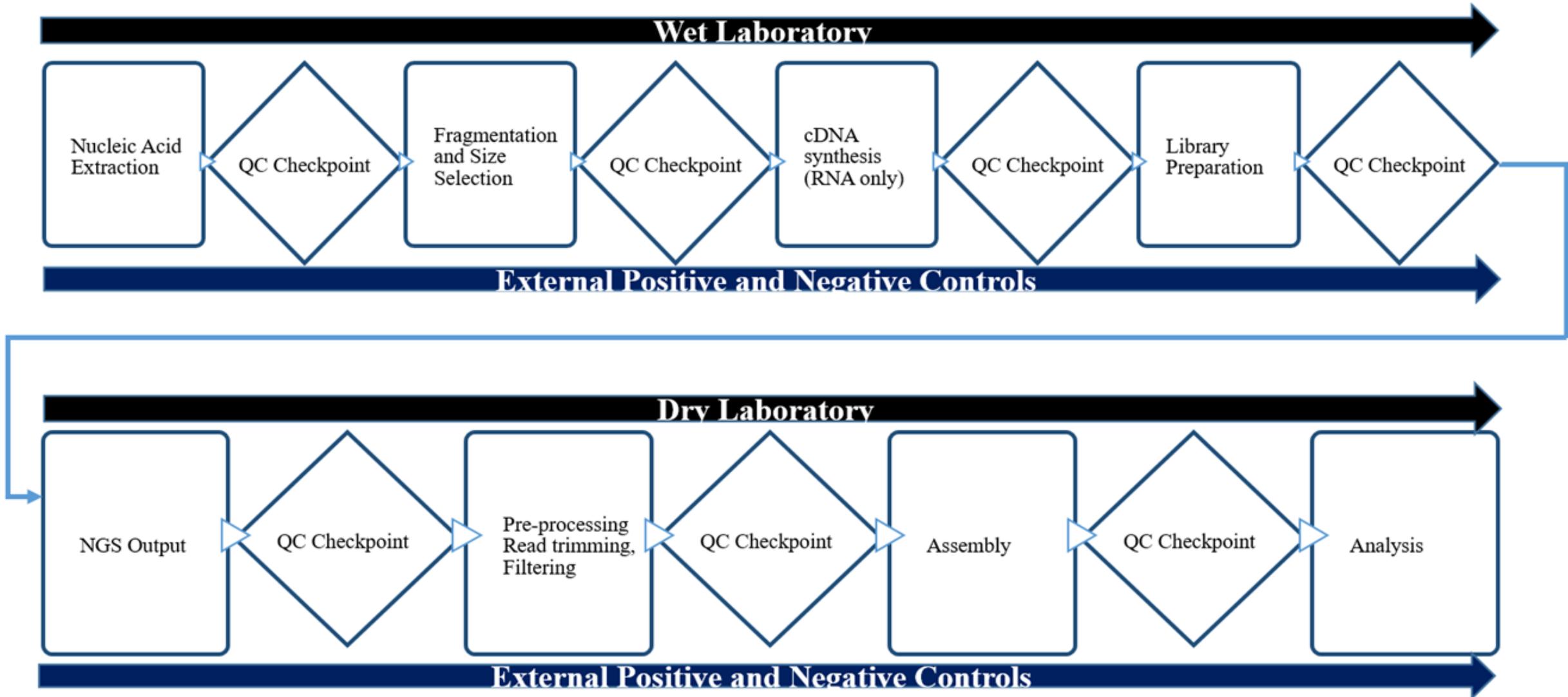
- NGS workflows contain several steps that need to be controlled for quality of inputs and outputs.
- An external control provides data at the end of the workflow and may not provide the information necessary to determine the success of individual sample sequencing.
- Quality control materials can be either specific to the assay or general to any sequencing workflow. However, general quality control materials may not provide information on the data analysis portion of the workflow.

CDC Public Health Laboratory Practices

- Quality Control checkpoints were implemented for each major step of the NGS workflow.
- Exploring IQCP mechanisms regarding frequency of external controls.

Continuing Challenge: Addressing gaps in guidance on the best way to control the NGS workflow, including-internal controls, external controls, and the flexibility to develop quality control metrics and checkpoints for the specific NGS workflow as methods and technologies change over time.

Process Control for the wet and dry NGS laboratories



Challenges and the Efforts to Address Challenges-III

Challenges of Distributive Testing

- Components of testing can be performed in different laboratories and under different CLIA certificates. Guidance is needed for:
 - Quality control criteria
 - Internal/external control criteria
 - Demonstrating proficiency of a module within the NGS workflow.
 - Determining responsibilities for using and/or hosting databases or analytic algorithms.

CDC Public Health Laboratory Practices

- Apply Quality Control checkpoints for incoming sequence data or extracted DNA.
 - PHL partners
 - Internally during surge testing responses
- Develop curated databases and make them available to partner laboratories.
- Move towards standardization of the sequence metadata stored in databases to make data comparison meaningful.

Continuing Challenge: Addressing gaps in guidance on internal and external controls when the NGS workflow spans laboratories, alternative assessments across laboratories. Lack of standardization regarding database submission requirements for information that should be linked to sequence data (e.g., platform, chemistries).

Challenges and the Efforts to Address Challenges-IV

System Validation and Re-validation

- NGS workflows contain several steps and use multiple sets of reagents so that new lot numbers are frequently introduced at various points in the workflow.
- Hardware and software are frequently updated.
- Improvements or updates to one part of the process can effect everything downstream.

CDC Public Health Laboratory Practices

- The initial validation is performed with a full set of samples to establish performance characteristics.
- A representative subset of the samples is selected to perform reproducibility testing during the initial validation.
- Any changes to the workflow are evaluated for risk to the results.
- Re-validation following changes determined to have low risk to the results (e.g., new DNA extraction method of similar chemistry) is performed using the representative subset of samples run through the entire workflow.

Continuing Challenge: As modules (e.g., library prep method, nucleic acid extraction method, sequence assembly tools) in the workflow are updated over time, there are gaps in guidance to standardize risk assessment and ensure the appropriate level of re-validation occurs.

Challenges and the Efforts to Address Challenges-V

Analysis and Reporting Challenges

- NGS testing provides a massive amount of data for epidemiologic public health use, but of limited diagnostic value.
- The amount of generated data often contrasts with best practices for patient diagnostic reports to be clear, concise and limited to critical information.
- Which records (data file and format) to retain is not well defined.
- Undefined obligation for re-analysis of previously tested specimens as databases and knowledge are updated.

CDC Public Health Laboratory Practices

- Maintain clear demarcation for testing that falls under CLIA for results that go back to patients or their providers.
- Ongoing evaluation of various records retention requirements and data storage infrastructural capacity.

Continuing Challenge: As surveillance and diagnostic workflows merge, continued thought is needed for what information is of diagnostic use to clinicians and patients. There is increasing need for record retention policies and secondary results reporting policies.

Summary of Challenges and Proposed Way Forward

- New technologies require new skills and it would benefit laboratories to have the flexibility to match personnel with the appropriate skill sets to the tasks performed within the NGS workflow.
- Laboratories would benefit from process controls designed for NGS workflows (and distributed NGS workflows) so that the controls mitigate the risks of this technology. We would like to engage with the broader community to design a control system for NGS methods from the ground up.
- Due to the complex and modular make up of NGS methods, laboratories would benefit from the flexibility to perform risk assessments to changes in the method, and then perform the appropriate level of verification/re-validation to mitigate that risk.
- Due to the large number of files generated during NGS data analysis, laboratories would benefit from record retention guidance on key file formats and information to be retained for two purposes: 1) re-analysis, and 2) supporting data for the original results.

Acknowledgements

- NGS Quality Workgroup Members
- NGS Quality Review Board Members

Questions

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